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Hydrogen peroxide is thought to regulate cellular processes by direct oxidation of numerous cellular proteins, whereas antioxidants, most notably thiol peroxidases, are thought to reduce peroxides and inhibit H_2O_2 response. However, thiol peroxidases have also been implicated in activation of transcription factors and signaling. It remains unclear if these enzymes stimulate or inhibit redox regulation and whether this regulation is widespread or limited to a few cellular components. Herein, we found that *Saccharomyces cerevisiae* cells lacking all eight thiol peroxidases were viable and withstood redox stresses. They transcriptionally responded to various redox treatments, but were unable to activate and repress gene expression in response to H_2O_2. Further studies involving redox transcription factors suggested that thiol peroxidases are major regulators of global gene expression in response to H_2O_2. The data suggest that thiol peroxidases sense and transfer oxidative signals to the signaling proteins and regulate transcription, whereas a direct interaction between H_2O_2 and other cellular proteins plays a secondary role.

Numerous cellular processes, including transcription and signaling, are redox regulated, but the molecular basis for this regulation is not clear. Hydrogen peroxide (H_2O_2) is a key molecule that is involved in redox regulation (1–3). It is both a toxic compound that can cause oxidative stress (4) and a second messenger that is required for cell proliferation (5). Its signaling function is thought to result from direct oxidation of various cell signaling and regulatory components, and its toxicity from stochastic oxidative damage to proteins, lipids, and nucleic acids (6, 7).

Several classes of enzymes, such as catalases and peroxidases, have evolved that specifically act on H_2O_2 or other hydroperoxides as substrates. Prominent among them are thiol-dependent peroxidases, which belong to peroxiredoxin (Prx) and glutathione peroxidase (Gpx) protein families. Thiol peroxidase genes are present in all previously characterized organisms, suggesting that these enzymes serve important functions conserved throughout evolution. Prx and Gpx have been implicated in cell signaling due to their ability to reduce intracellular levels of hydroperoxides and to serve as floodgates of H_2O_2 signaling (8–10). However, studies have also revealed that *Saccharomyces cerevisiae* Gpx3/Hyr1/Orp1 can serve as an H_2O_2 sensor and activate the transcription factor Yap1 by forming a disulfide in this protein (11), and a *Schizosaccharomyces pombe* thiol peroxidase Tsa1 was found to stimulate signaling through a MAP kinase pathway (12, 13). *S. pombe* thiol peroxidase Tpx1 similarly regulates transcription factor Pap1 (14). In addition, the ability to transfer oxidizing equivalents was demonstrated for a mammalian GPx4 using a GFP-based redox sensor (15). It would be important to address the contribution of thiol peroxidases to stimulation and repression of redox regulation, particularly at a global, genome-wide scale.

In this work, we prepared a *S. cerevisiae* mutant lacking all eight thiol peroxidases. Surprisingly, this mutant was viable and could withstand significant oxidative stress. It responded to several redox stimuli by robust transcriptional reprogramming. However, it was unable to transcriptionally respond to hydrogen peroxide. The data suggested that thiol peroxidases transfer oxidative signals from peroxides to target proteins, thus activating various transcriptional programs. This study revealed a previously undescribed function of these proteins, in addition to their roles in removal of low levels of peroxides.

Results and Discussion

Yeast Cells Lacking All Thiol Peroxidases Are Viable and Can Withstand Redox Stresses. *S. cerevisiae* has five peroxiredoxins (Tsa1, Tsa2, Ahp1, nPrx, and mPrx) (16) and three glutathione peroxidases (Gpx1, Gpx2, and Gpx3) (17). We generated a series of mutants lacking multiple thiol peroxidases in different combinations (Fig. S1 and Table S1). These included several mutants that lacked seven (7Δ; three mutants with remaining Gpx2, Gpx3, or Tsa1) or all eight (8Δ) thiol peroxidase genes. The genome of the 8Δ strain was sequenced to 26.5× coverage on an Illumina platform, and the disruption of all eight thiol peroxidase genes was confirmed by DNA sequence analysis. All mutants lacking multiple thiol peroxidases, including 8Δ, were viable, although their growth was affected compared to WT cells (Fig. 1*A* and Fig. S2). The mutant cells could withstand treatments with significant amounts of H_2O_2, diamide, DTT, and menadione, although some mutants were more sensitive than parental (WT) cells to these redox stresses (Fig. 1*A* and Fig. S2). Individual thiol peroxidases differentially contributed to this protection, with cells lacking multiple thiol peroxidases generally being more sensitive to stress. Removal of all eight thiol peroxidases also decreased cell growth in the absence of stressors (Fig. S2). We further compared viability of WT and multiple thiol peroxidase mutant cells following treatment with 1 or 2 mM H_2O_2, 3 mM diamide, or 25 mM DTT for 0.5–2 h and found smaller differences between WT and mutant strains (Fig. 1*B*). This observation suggests that the growth of 8Δ cells was inhibited by acute oxidative stress (Fig. 1*A* and Fig. S2), but cells were viable and could resume growth once stressors were removed (Fig. 1*B*). The unexpected oxidative stress resistance of 8Δ cells could be explained by the protective activities of catalase and cytochrome c peroxidase, which can remove hydrogen peroxide in thiol peroxidase mutant cells. Progressive

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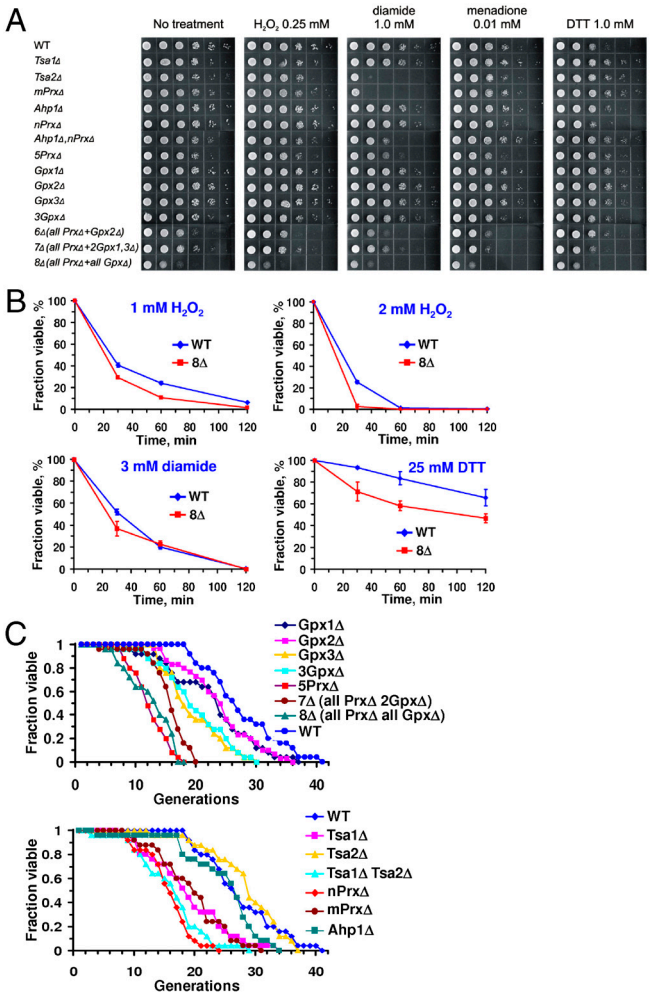

Fig. 1. Phenotypes and sensitivity of thiol peroxidase mutant strains to redox stresses. (*A*) Sensitivity of *S. cerevisiae* WT and different thiol peroxidase mutant cells to indicated concentrations of hydrogen peroxide, diamide, DTT, and menadione. Cells in series of 10× dilutions for each strain were grown on plates with indicated stress inducers for 48 h. (*B*) Viability of WT cells and 8Δ cells during 2-h treatment with indicated stressors. (*C*) Replicative life span of WT and thiol peroxidase mutant strains.

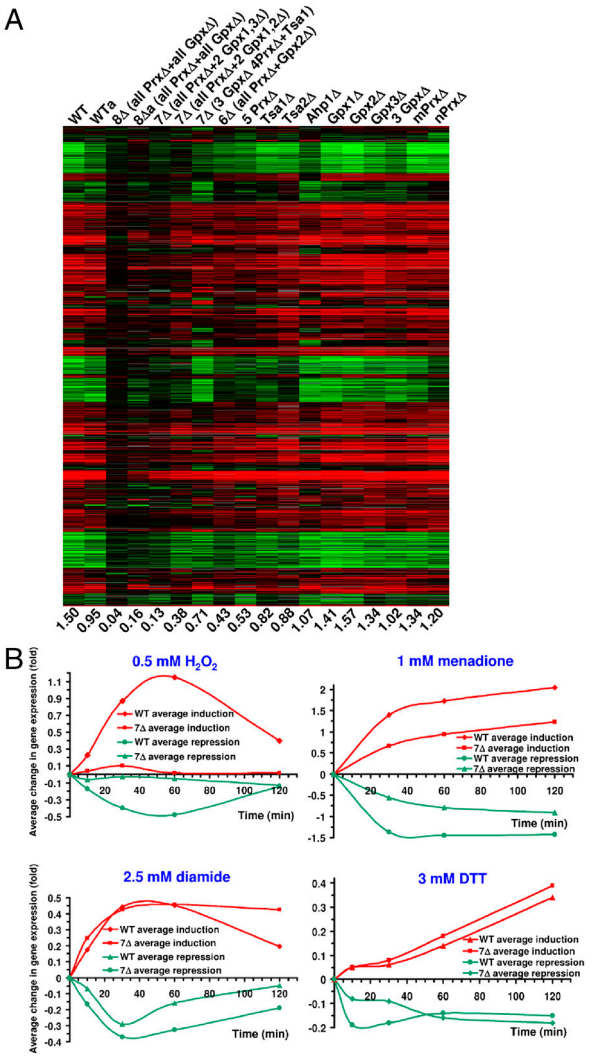

Fig. 2. Disruption of H_2O_2-dependent regulation of gene expression in yeast cells lacking multiple thiol peroxidase genes. (*A*) Regulation of gene expression by H_2O_2. Changes in gene expression of WT and indicated mutant thiol peroxidase strains in response to 0.5 mM H_2O_2 treatment (30 min) are shown for all genes that are either induced or repressed in at least one strain (WT or mutant) used in the study. Numbers below the columns show average values of changes in gene expression (the sum of activation and repression values divided by the total number of yeast genes as described in *Materials and Methods*) for each dataset. WTa and 8Δa refer to the corresponding H_2O_2-treated and untreated cells grown under anaerobic conditions. Repression is indicated by green and induction by red colors, and their intensities are graded as log2 of the fold increase/decrease in gene expression. (*B*) Time course of average changes in gene expression in WT and 7Δ cells in response to 0.5 mM H_2O_2, 2.5 mM DTT, 2.5 mM diamide, and 1 mM menadione treatments.

removal of thiol peroxidases also resulted in lower life span (Fig. 1*C*). Taken together, these data suggested that the loss of thiol peroxidases decreased cell fitness and affected redox homeostasis. However, these enzymes were not essential, even when cells were treated with peroxide or other stressors.

Thiol Peroxidase Null Cells Are Unable to Activate and Repress Gene Expression in Response to H_2O_2. In *S. cerevisiae*, a large number of genes respond to H_2O_2 (18). Using cDNA microarrays with 6,692 gene features, we found that expression of 1,144 genes was induced and 574 genes repressed more than 2-fold upon incubation of WT cells with 0.5 mM H_2O_2 for 30 min (Fig. 2*A*). On average, gene expression was changed in WT cells 1.5-fold under these conditions (Fig. 2*A* and Fig. S3).

The main known function of thiol peroxidases is to scavenge hydroperoxides. Accordingly, these proteins may be expected to decrease the transcriptional response to H_2O_2 by lowering the cellular peroxide levels. Therefore, deletion of the peroxidase genes would be expected to stimulate gene expression in response to H_2O_2. However, in contrast to this prediction, the response to H_2O_2 was inhibited in cells lacking multiple thiol peroxidases. This effect was especially pronounced in the mutants that lacked six or more thiol peroxidase genes (Fig. 2*A* and Fig. S3) and was observed at both 0.1 and 0.5 mM H_2O_2 (Fig. S4). Moreover, cells lacking seven (i.e., all except Gpx2, all except Gpx3, and all except Tsa1) or all eight thiol peroxidases essentially lost the ability to regulate gene expression in response to H_2O_2. For example, 8Δ cells had a 37-fold reduced response to H_2O_2 treatment compared to WT cells. Importantly, both activation and repression of gene expression were inhibited (Fig. 2*A* and Fig. S3).

Thiol Peroxidase Null Cells Transcriptionally Respond to Stresses Other Than H_2O_2. To determine if the observed transcriptional effect was specific to H_2O_2 or if the mutant cells also lost the ability to respond to other stresses, we examined the response of WT and 7Δ (all thiol peroxidases except Gpx2) cells to several other redox stressors, including DTT, diamide, and menadione (Fig. 2*B*). DTT is a strong reductant that causes reductive stress and induces

unfolded protein response (18), whereas menadione is a superoxide generator, and diamide is an oxidant that generates nonspecific disulfide bonds. WT cells responded to all treatments. We also found that 7Δ cells responded to DTT and diamide similarly to WT cells; however, they did not respond to H_2O_2 treatment. Thus, deletion of multiple thiol peroxidase genes specifically disrupted the H_2O_2-induced transcriptional reprogramming without affecting the ability of cells to respond to other redox stresses. The response of mutant cells to menadione was twofold lower than that in WT cells (Fig. 2*B*), but this effect could be explained by the fact that this compound generates superoxide, which is further converted to H_2O_2 (to which these cells do not respond).

Thiol Peroxidase Null Cells Do Not Respond to Varying H_2O_2 Concentrations and Treatment Times. To examine the possibility that the response to H_2O_2 in the thiol peroxidase mutants was delayed or accelerated rather than the regulation was abolished, we analyzed gene expression in WT and 7Δ cells at 10, 30, 60, and 120 min after addition of H_2O_2 (Fig. 2*B*). In WT cells, gene expression (both activation and repression) changed by 30 min, peaked at 60 min, and diminished at 120 min. However, 7Δ cells did not respond to H_2O_2 at any time points. Likewise, we examined the regulation of gene expression in WT and 8Δ cells at different concentrations of H_2O_2 (0.05, 0.5, 1, 2, and 5 mM; 30-min treatment) (Fig. S5). In WT cells, activation and repression were most pronounced at 0.5 mM H_2O_2, but at concentrations above 1 mM the response diminished (Fig. 1*B*). However, the response of 8Δ cells was low at any H_2O_2 concentration. To test if other redox proteins functionally linked to thiol peroxidases were involved in the H_2O_2 response, we examined yeast cells deficient in cytochrome c peroxidase (Ccp1) or sulfiredoxin (Srx1). Both mutants responded to H_2O_2 similarly to WT cells (Fig. S6).

The data presented so far are consistent with a model wherein thiol peroxidases were required for the transfer of the H_2O_2 signal to other cellular components for transcriptional reprogramming. Moreover, this requirement was not limited to certain gene groups. Thus, thiol peroxidases appeared to function as global mediators (rather than inhibitors) of gene expression in response to H_2O_2.

Thiol Peroxidase-Dependent Repression of Ribosomal Protein Gene Expression. As a representative example, we analyzed expression of cytosolic ribosomal protein genes in WT and thiol peroxidase mutant cells. Ribosomal protein genes (*i*) were not down-regulated by H_2O_2 in mutant cells (Fig. 3*A*), (*ii*) exhibited normal expression levels in untreated mutant cells compared to WT cells (Fig. 3*B*), (*iii*) were down-regulated by H_2O_2, diamide, and DTT in WT cells, and (*iv*) were down-regulated by DTT and diamide (but not by H_2O_2) in mutant cells (Fig. 3*C*). These data argue for the specific thiol peroxidase-dependent regulation of ribosomal protein gene expression.

Expression of ribosomal protein genes is regulated by transcription factor Sfp1 (19). We tested the response of Sfp1 mutant cells to H_2O_2 (Fig. 3*D*) and found no repression of ribosomal protein genes, suggesting that Sfp1 is a H_2O_2- and thiol peroxidase-regulated transcription factor. An additional related cluster of genes that failed to respond to H_2O_2 treatment in 8Δ cells includes genes involved in rRNA modification and translation (Figs. S7 and S8). Similar to cytosolic ribosomal protein genes, rRNA modification and translation genes were not activated or repressed in response to H_2O_2. However, their responses to DTT and diamide treatments in mutant cells were similar to those in WT cells. Interestingly, although cytosolic translational machinery was transcriptionally repressed by H_2O_2 in a thiol peroxidase-dependent manner (Fig. 3 and Figs. S7 and S8), mitochondrial ribosomal protein genes were induced (Fig. S9). A similar effect was observed for genes coding for ubiquitin-dependent protein degradation components (Fig. S10). Further

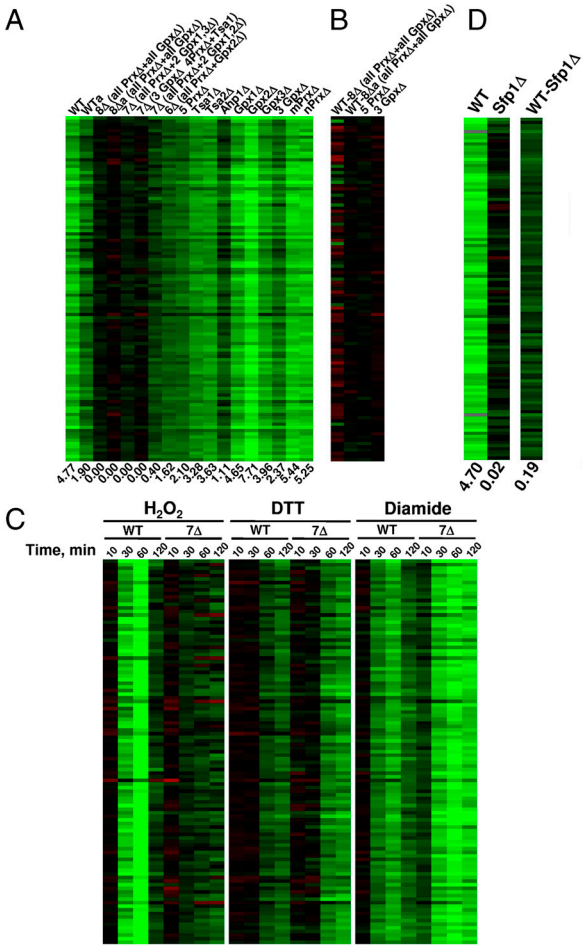

Fig. 3. Thiol peroxidase-dependent transcriptional regulation of ribosomal proteins by H_2O_2. (*A*) Changes in gene expression of cytosolic ribosomal proteins upon treatment of WT and thiol peroxidase mutant cells with 0.5 mM H_2O_2 for 30 min. (*B*) Changes in gene expression of cytosolic ribosomal proteins between untreated WT and mutant cells. (*C*) Time course of gene expression changes of cytosolic ribosomal proteins upon treatment of WT and 7Δ cells with 0.5 mM H_2O_2, 2.5 mM diamide. or 2.5 mM DTT for indicated time periods. (*D*) Changes in gene expression for cytosolic ribosomal proteins upon treatment of WT and Sfp1 mutant cells with H_2O_2. Right column compares gene expression in WT and Sfp1Δ cells.

examination of responses in individual thiol peroxidase mutants suggested that thiol peroxidases could compensate for each other in mediating the repression of cytosolic ribosomal and translation-related proteins. Nevertheless, the contributions of individual thiol peroxidases to the peroxide-dependent regulation varied (Fig. 3*A* and Fig. S8).

Thiol Peroxidase Null Cells Do Not Show Elevated Levels of Reactive Oxygen Species (ROS). Thiol peroxidases have been suggested to serve as key enzymes in antioxidant defense. If so, a possibility had to be considered that the deletion of these enzymes led to oxidative stress (and therefore resulted in the H_2O_2 response even in the absence of stress) and that treatment with H_2O_2 did not further change or exacerbate the expression profile or response. Because the definition of oxidative stress is complex, we examined this possibility in a number of ways, as described in several sections below. First, we analyzed ROS levels in WT and mutant cells by monitoring 2′,7′-dichlorofluorescein (DCF) fluorescence (Fig. 4*A*). Little difference was found between WT and 8Δ cells in the absence of stress; however, ROS levels were 2-fold higher in multiple thiol peroxidase mutant cells following 1–5 mM H_2O_2 treatment (Fig. 4*A*). These data suggest that 8Δ

cells were not in a state of severe peroxide stress that could preclude their response to H_2O_2 treatment. The increase in ROS levels in 8Δ cells after the addition of H_2O_2 could not explain the loss of the transcriptional response because 8Δ cells could transcriptionally respond to other stresses (Fig. 2*B*). In addition, in the presence of 0.1 mM H_2O_2, ROS were not increased in 8Δ cells, yet transcriptional response was inhibited (Fig. S4).

Thioredoxin and Thioredoxin Reductase Null Cells Respond to H_2O_2 Treatment. The reduced state of most or all thiol peroxidases in the yeast cytosol and nucleus is maintained by thioredoxins Trx1 and Trx2, which in turn are reduced by NADPH-dependent thioredoxin reductase Trr1. We tested if deletion of both Trxs or of Trr1 also disrupts regulation of gene expression by H_2O_2, but instead found that these mutants had an increased response to H_2O_2 (Fig. 4*B*). Stimulation of activation and repression of gene expression in response to H_2O_2 was also previously seen in the Trr1 mutant cells treated with lower concentrations of H_2O_2 (20). Thus, we observed an important difference between thiol peroxidases on the one side and their reductants on the other. The data suggest that deletion of Trxs or Trr1 leads to accumulation of oxidized forms of thiol peroxidases and therefore results in an increased response to H_2O_2.

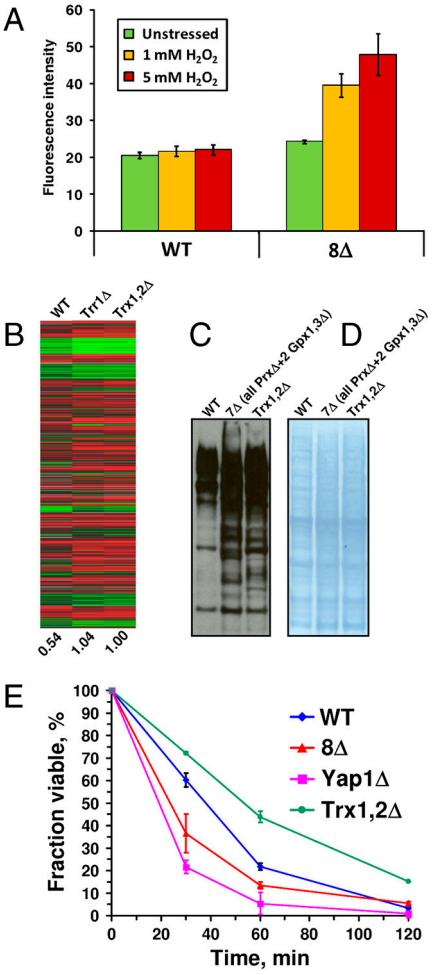

Fig. 4. ROS in thiol peroxidase mutant cells and the peroxide response in thioredoxin mutant strains. (*A*) ROS in WT and 8Δ cells were assayed by DCF fluorescence. Cells were treated with indicated concentrations of peroxide for 30 min. (*B*) Changes in gene expression in WT, Trx1,2Δ. and Trr1Δ cells in response to H_2O_2 treatment. Yeast strains in SY2626 background were used in this experiment. (*C*) Analysis of oxidized cysteine residues in yeast proteins in WT and indicated mutant strains. Reduced cysteine residues in yeast proteins were blocked with iodoacetamide, and the oxidized residues were reduced with DTT, alkylated with a biotinylated iodoacetamide, and visualized with streptavidin-conjugated antibodies. (*D*) Protein loading control for the data shown in *C*. (*E*) Viability of WT, 8Δ, Yap1Δ, and Trx1,2Δ cells following treatment with 1 mM H_2O_2 for indicated periods of time.

Thioredoxin and Thiol Peroxidase Mutants Show Similar Levels of Cysteine Oxidation. Because Trxs, Trr1, and thiol peroxidases function in the same pathway to transfer electrons from NADPH to H_2O_2, further comparison of these mutant strains offered us an opportunity to better understand the unique role of thiol peroxidases in the peroxide-dependent transcriptional regulation. We examined levels of oxidized cysteine residues in protein extracts from unstressed 7Δ and Trx1,2Δ cells. Although both strains had somewhat elevated cysteine oxidation compared to WT cells, we found no significant differences between these mutant cells (Fig. 4 *C* and *D*). These data further support the idea that redox stress cannot explain the specific block in the H_2O_2 response by thiol peroxidase null cells.

During the course of these studies, we made another interesting observation: Trx1,2Δ cells were more resistant to H_2O_2 treatment in the cell viability assay than even WT cells (Fig. 4*E*). It is possible that Trx1,2Δ cells had an increased capacity for H_2O_2 regulation due to elevation in oxidized thiol peroxidases. This finding is not contradictory with the current literature (20, 21). Indeed, the double thioredoxin mutant grows slowly in the presence of H_2O_2; however, its viability, determined as the number of colonies formed following the treatment and transfer of cells to a new medium, was not affected. Overall, our data support the idea that Trr1 and Trxs inhibit the transcriptional response to H_2O_2 (by reducing thiol peroxidases), whereas thiol peroxidases stimulate peroxide-dependent regulation of gene expression (by oxidizing target proteins).

Antioxidant Compounds and Anaerobic Growth Do Not Restore the Ability of 8Δ Cells to Respond to Peroxide. As an additional test, we examined the H_2O_2-dependent changes in gene expression of WT and 8Δ cells under anaerobic conditions (Fig. 2*A*), as well as aerobically in the presence of antioxidant compounds, 5 mM L-proline and 5 mM N-acetylcysteine (NAC) (Fig. S11). The difference in gene expression between WT and 8Δ cells (Fig. S11, *Right*) was significantly decreased by anaerobiosis and proline/NAC treatment; however, these conditions and treatments did not restore the ability of thiol peroxidase mutant cells to respond to H_2O_2. These data once again argue that peroxide stress is not a reason for the inability of the mutant strains to respond to H_2O_2.

Antioxidants Inhibit Rather Than Stimulate Growth of 8Δ Cells. We tested if expression of a bacterial thiol peroxidase, *Escherichia coli* 2-Cys peroxiredoxin BCP (Fig. S12), or treatment of the thiol peroxidase mutant strain with 5 mM NAC (Fig. S13) could decrease the elevated cysteine oxidation in multiple thiol peroxidase mutants and found that they could not. In addition, we examined if antioxidants (5 mM NAC and 2 mM DTT) could normalize the growth of thiol peroxidase mutant cells. Surprisingly, the growth of 8Δ cells was inhibited by both compounds (Fig. S14). For example, whereas the growth of WT cells was not affected by NAC, the NAC-treated 8Δ cells showed no growth until 20 h. Moreover, 8Δ cells did not grow in the presence of 2 mM DTT (Fig. S14), even though this compound did not affect viability of these cells (during brief exposure) to much higher concentrations (Fig. 1*B* and Fig. S2). Although 5 mM NAC did not change the overall levels of disulfides in WT and mutant cells (Fig. S13), our observations support the idea that DTT- and NAC-dependent inhibition of growth of 8Δ cells was due to the reduction of disulfides in regulatory proteins. It is possible that the increased levels of nonspecific cysteine oxidation in multiple thiol peroxidase mutant cells is a molecular response that protects cells from stress caused by deletion of thiol peroxidase genes.

Roles of Yap1, Skn7, Msn2, and Msn4 in the Global Response to H_2O_2. In *S. cerevisiae*, four transcription factors, Yap1, Skn7, Msn2, and Msn4, are known to regulate gene expression in response to H_2O_2, but they can also be activated by other stresses. We deleted these genes individually or in combination and tested the transcriptional response of the resulting cells to H_2O_2. These mutant cells, including the mutant that lacked all four transcription factors (4Δ), were viable and responded to H_2O_2 similarly to WT cells (Fig. 5*A* and Fig. S15); however, sensitivity to H_2O_2 was higher in Yap1Δ and 4Δ cells compared to WT cells. For example, expression of ribosomal protein genes was inhibited by H_2O_2 in these cells (Fig. S16). Comparison of expression profiles between WT and cells lacking the four transcription factors showed little difference (Fig. 5*B*). Reversible cysteine oxidation in the mutant cells was also similar to that in WT cells under equivalent treatment conditions (i.e., with and without H_2O_2) (Fig. S17). These results suggest that the global response to H_2O_2 is not mediated exclusively by these four redox transcription factors. Our data also allowed us to better define the sets of genes dependent on these transcription factors (Fig. 5 *C* and *D*). In particular, we identified genes uniquely dependent on Yap1 as well as genes dependent on both Yap1 and Skn7 (Fig. 5*C*).

Expression of several, but not all, Yap1- and Yap1/Skn7-dependent genes was elevated in the untreated 8Δ cells compared to WT controls (Fig. 5*D*). It is known that Yap1 can be activated by general stresses, including treatment with diamide, and this regulation may be independent of thiol peroxidases (22). We examined changes in the expression of Yap1- and Yap1/Skn7-dependent genes in WT and 7Δ cells upon treatment with diamide, menadione, or DTT at different time points (Fig. S18). The analysis of the response to menadione and diamide suggested that Yap1/Skn7 dependent genes could still be fully regulated in 7Δ cells. On the other hand, the DTT response showed an opposite effect for WT and 7Δ cells. This observation suggested that disulfide bonds in Yap1/Skn7 transcription factors may have formed nonspecifically in the oxidative environment of 7Δ cells and that such disulfide bonds could not be reoxidized under experimental conditions, because formation of physiological disulfide bonds was not possible in the 7Δ mutant.

Hydrogen Peroxide-Sensing Cysteines Are Intact in 8Δ Cells. We tested the response of WT and 8Δ cells to the combined H_2O_2 and diamide treatment. H_2O_2 did not modify the response of WT and 8Δ cells to diamide (Fig. S19). Moreover, transcriptional responses to H_2O_2 and diamide significantly overlapped in WT cells and the response to H_2O_2 in WT cells also overlapped with the diamide response in 8Δ cells (Figs. S19, S20, and S21). Diamide can form disulfide bonds in proteins nonspecifically, and this observation explains the overlap between H_2O_2 and diamide responses and suggests that both compounds have a similar set of targets. These data support the hypothesis that 8Δ cells do not respond to H_2O_2 because they lack thiol peroxidases, which form regulatory disulfides in signaling proteins. Diamide can directly form such disulfides, and therefore the response to diamide in 8Δ cells is similar to those of H_2O_2 and diamide in WT cells.

A Model of Thiol Peroxidase-Dependent Regulation of Transcription. We showed that thiol peroxidase null cells are unable to sense H_2O_2 and carry out peroxide-dependent transcriptional reprogramming. The peroxide response was not observed at any time points following peroxide treatments nor at any peroxide concentrations. Neither anaerobic conditions nor antioxidants could restore it. Yet, thiol peroxidase mutant cells showed robust transcriptional responses to other redox treatments. We propose that these findings can be explained by disruption of the signaling network from peroxide to transcription factors. Thiol peroxidases emerge as global regulators of gene expression. Overall, the data suggest that thiol peroxidases exhibit two functions associated

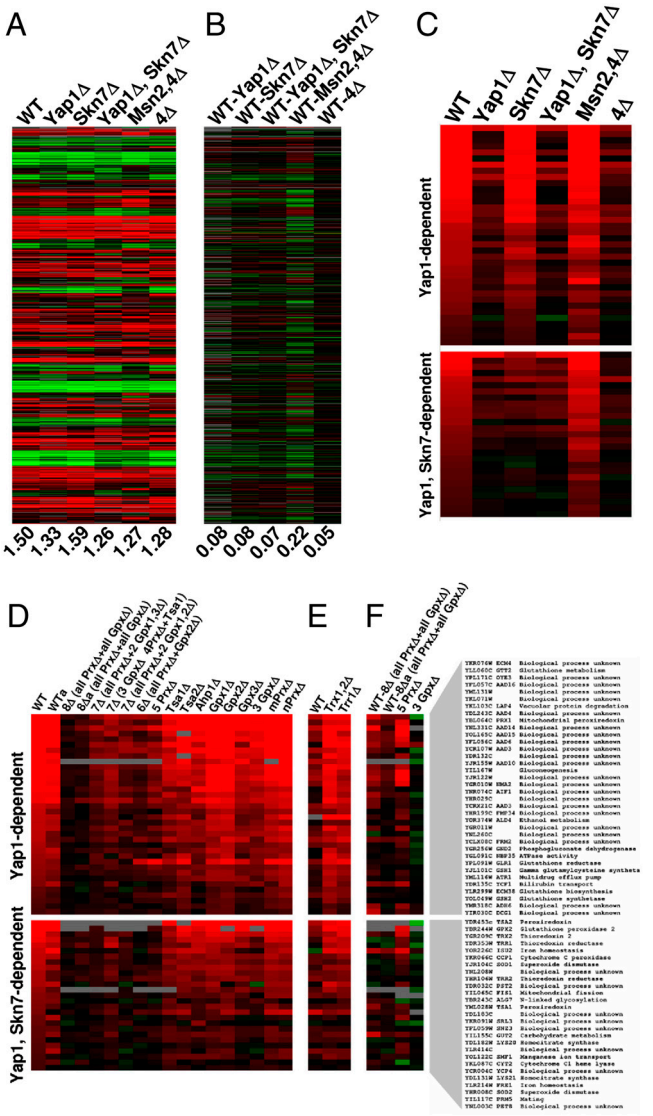

Fig. 5. Role of oxidative stress transcription factors in the H_2O_2 response. (*A*) Regulation of gene expression by H_2O_2 in cells lacking single and multiple transcription factors Yap1, Skn7, Msn2, and Msn4. (*B*) Comparison of gene expression between untreated WT and indicated mutant cells. The values of average changes in gene expression are shown under each column. (*C*) Changes in gene expression in response to H_2O_2 treatment of Yap1- and Yap1/Skn7-dependent genes in WT and transcription factor mutant cells. This set was generated by filtering the entire set of genes responsive to H_2O_2 treatment as described in *Materials and Methods*. (*D*) Changes in gene expression, in response to H_2O_2 treatment of Yap1- and Yap1/Skn7-dependent genes, in WT and thiol peroxidase mutant cells. *D* shows a filtered version of data from Fig. 2*A*. (*E*) Same experiment performed with Trr1Δ and Trx1,2Δ cells and isogenic WT cells. *E* shows a filtered version of data from Fig. 4*B*. (*F*) Changes in gene expression of Yap1- and Yap1/Skn7-dependent genes between WT and indicated mutant cells. Yap1- and Yap1/Skn7-dependent genes are shown on the right.

with H_2O_2 reduction: (*i*) They transmit oxidative signals to upstream (with respect to electron flow) effectors, such as transcription factors and signaling and regulatory molecules, and (*ii*) they provide antioxidant defense by reducing hydroperoxides. Previously, it has been difficult to distinguish these functions experimentally because by reducing peroxides, thiol peroxidases fulfill two roles: oxidation of target proteins and antioxidant defense.

Our model of gene regulation by H_2O_2 (Fig. 6) is illustrated using the example of the NADPH-dependent Trx system. In the absence of stress, thiol peroxidases are kept in the reduced state by Trxs and other thiol-disulfide oxidoreductases. Upon addition

(or intracellular generation) of low concentrations of H_2O_2, this compound specifically oxidizes thiol peroxidases, whereas oxidation of other cellular proteins by H_2O_2 is minimal. High specificity of these enzymes toward H_2O_2 (23) makes them excellent candidates for sensing H_2O_2 and other hydroperoxides and allows them to effectively compete for H_2O_2 with other proteins containing reactive cysteines. Thiol peroxidases then oxidize regulatory and signaling proteins, resulting in the transcriptional response and signaling programs. The examples of Gpx3-dependent activation of Yap1 in response to H_2O_2 (11, 24) and of Tsa1-dependent activation of a stress-activated MAP kinase (12, 13) are supportive of our model and illustrate a molecular mechanism, by which thiol peroxidases may transfer oxidative signals to regulate gene expression.

Although regulation due to a direct target oxidation by H_2O_2 also likely exists, the key difference between our model (Fig. 6) and previously suggested models of redox regulation is that H_2O_2 does not need to interact with other cellular proteins to a significant extent in order to regulate gene expression or other peroxide-dependent programs. The model also explains specificity of H_2O_2 transcriptional regulation and points to basic mechanisms of redox signaling and redox regulation of cellular processes. Finally, we found that thiol peroxidases regulate the H_2O_2 transcriptional response in *S. cerevisiae*. We suggest that thiol peroxidases, via thiol-based redox coupling with cellular proteins, may also control different signaling and regulatory programs in other organisms, including mammals.

Materials and Methods

A detailed *SI Materials and Methods* is located in *SI Appendix*.

Yeast Strains. The yeast strains used in this study are shown in Table S1. Single and multiple thiol peroxidase mutants were prepared by mating the strain lacking five peroxiredoxin genes (GY14) with the strain lacking three glutathione peroxidase genes (GY25, GY30) in BY4741 background. The transcription factor mutant strains were prepared by a one-step gene disruption method.

Spot Assays. Overnight cultures were adjusted to $OD_{600} = 1$, and 10 μL of serial dilutions (10-fold each) were spotted on SD solid medium that contained stressors at indicated concentrations. Cells were grown for 2 d.

Yeast Aging Assays. After growing for 2 d on fresh plates, 35 undivided daughter cells were collected and arranged on yeast YPD plates using a dissecting microscope. New buds from these original daughter cells were separated and discarded as they formed. This process continued until cells stopped dividing.

cDNA Microarray Analyses. Cells were grown to 0.3–0.5 OD_{600} in 200 mL of YPD medium, treated for indicated times with indicated compounds, harvested by centrifugation, and kept at −80 °C. Total RNA was isolated, and mRNA was prepared by amplification and used to prepare cDNA probes by reverse transcription with incorporation of Cy3-dCTP or Cy5-dCTP. DNA microarray data were K-mean clustered with CLUSTER and visualized using TreeView. Average levels of gene activation and repression were estimated as described in *SI Materials and Methods*.

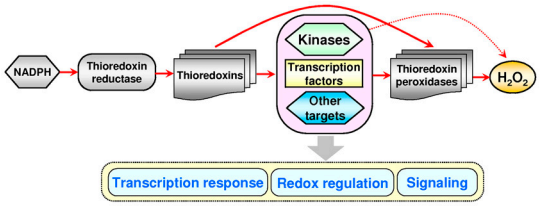

Fig. 6. A model of redox regulation of gene expression. Shown is the flow of reducing equivalents (red arrows) in the thioredoxin system from NADPH to hydrogen peroxide and the role of thiol peroxidases in this process. Thiol peroxidases are initially oxidized by H_2O_2 and then oxidize transcription factors, kinases, and other target proteins in yeast cells. Oxidation of these targets elicits transcriptional response, redox regulation, signaling pathways, and other programs (shown by a vertical gray arrow). Direct oxidation of signaling and regulatory proteins by H_2O_2 is minimal (red dotted arrow).

Detection of Reversibly Oxidized Cysteine Residues in WT and Mutant Yeast Strains. Each strain was grown in YPD to $OD_{600} = 1$. Reduced cysteines were modified with iodoacetamide under denaturing conditions. Then, remaining oxidized cysteines were reduced with DTT and modified with biotinylated iodoacetamide. The levels of oxidized cysteines (in the initial samples) were detected by Western blotting using streptavidin-conjugated antibodies.

Viability Assays. Each strain was grown in YPD to $OD_{600} = 0.5$ and treated with indicated stress agents for various time periods. Cells were washed with YPD medium and serial dilutions were plated on YPD agar plates. Colony numbers were counted after 3 d.

ROS Analyses. Yeast cells were grown to $OD_{600} = 0.5$, washed twice with PBS, resuspended in PBS to 10^8 cells/mL, and loaded with 5 μM 2′-7′-dichlorodihydrofluorescein diacetate. Cells were treated with 1 mM or 5 mM H_2O_2 for 30 min. DCF fluorescence was analyzed by flow cytometry.

Genome Sequence of 8Δ Cells. Genomic DNA was isolated from 8Δ cells and sequenced on an Illumina platform. Reads were assembled into the genome with MAQ genome assembler.

ACKNOWLEDGMENTS. We thank S. V. Avery (University of Nottingham, Nottingham, United Kingdom) and G. F. Merrill (Oregon State University, Corvallis, OR) for providing yeast strains, A. Bird for help with microarrays, and the University of Utah Microarray Core Facility for excellent technical assistance. This work was supported by National Institutes of Health (NIH) Grant GM065204 (to V.N.G.). J.C.R. was supported by NIH Grant GM083292 (to D.R.W.).

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Supporting Information

Materials and Methods

Strains, cells growth, and media. *S. cerevisiae* parental strain (referred to as WT) BY4741 (MAT**a** *his3Δ leu2*Δ *met15*Δ *ura3*Δ) and its isogenic thiol peroxidase mutants were grown in YPD media (1% yeast extract/2% peptone/2% dextrose). SY2626 cells were used as a parental strain for Trx1,2 and Trr1 mutant strains.

Preparation and verification of thiol peroxidase mutant stains. The yeast strains used in this study are shown in Table S1. GY15 is a MATα derivative of BY4741. The mutant missing five peroxiredoxin genes (GY14) in BY4741 background was previously described (1). To isolate single peroxiredoxin mutants, GY15 and GY14 were mated and diploids were sporulated and dissected by a micromanipulator. Spores that were missing Tsa1, Tsa1, mPrx, nPrx and Ahp1 genes were selected on supplemented YNB minimal media by auxotrophic markers that were used to replace the original ORFs. The single and triple glutathione peroxidase mutants were previously described (2).

In order to isolate a mutant (GY29) missing seven peroxidase genes (it expresses a single thiol peroxidase Gpx2), GY14 and GY30 were mated and the resulting diploids were analyzed by tetrad dissection. GY30 is an isogenic strain of GY25 that was provided by Dr. S. Avery (U. Nottingham, UK). At the first stage of screening, several hundred spores were checked on YNB selection plates for histidine, leucine, uracil and methionine auxotrophy and for G418 resistance. The mutant missing all 8 genes (8Δ, GY40) was generated from GY29 by a one-step gene disruption method using a hygromycin marker and pAG32 (3). Since each of HIS3, URA3 and KAN markers were used to delete 2 genes in the GY29 mutant cells, we performed a second screening to confirm both the deletion of each ORF and presence of locus-specific marker genes by PCR with amplification control for each strain used in our work. The presence/absence of a thiol peroxidase gene was also verified by quantifying microarray signals. The mutant lacking all eight thiol peroxidases was used to generate strains missing seven peroxidases genes by mating it with WT and selecting for cells containing single Gpx3 (GY110) and Tsa1 (GY111) thiol peroxidase genes.

A mutant strain missing both *yap1* and *skn7* (GY73) was prepared from GY70 *Δyap1:KAN* strain by a one-step gene disruption method using the *LEU2* marker. The strain missing *msn2* and *msn4* (GY74) was similarly prepared from GY72 *Δmsn2:KAN* using the *URA3* marker. The quadruple *Δyap1:KAN Δskn7:LEU2, Δmsn2:HIS3 Δmsn4:URA3* mutant (GY75) was generated from GY73 by two consecutive gene disruption steps using *msn2* (*HIS3* marker) and *msn4* (*URA3* marker).

Spot assays. Resistance of WT and mutant strains to different stressors was determined with spot assays. For each strain, overnight cultures were adjusted to OD_{600}=1 and 10 μl of serial dilutions (10-fold each) were spotted on SD solid medium that contained H_2O_2, diamide, menadione, or DTT at indicated concentrations. Cells were grown for two days at 30 °C and pictures were taken.

Yeast aging assays. After growing cells for two days on fresh plates, 35 undivided daughter cells were collected and arranged on YPD plates using a dissecting microscope. New buds from

these original daughter cells were separated and discarded as they formed. This process continued until cells stopped dividing. Lifespan was calculated as the total number of daughter cells that each cell generated. The aging experiment was performed twice for each strain and representative mortality curves are shown.

cDNA microarray analyses. Cells were grown to 0.3-0.5 OD_{600} in 200 ml of YPD medium, treated for indicated times with indicated compounds, harvested by centrifugation, and kept at -80 °C. Total RNA was isolated by Ambion RiboPure™-Yeast Kit according to the manufacturer's instructions. Antisense RNA, prepared by Ambion MessageAmp™ aRNA Amplification Kit, was used to prepare cDNA probes by reverse transcription with incorporation of Cy3-dCTP or Cy5-dCTP (Amersham Biosciences). The cDNA-labeled probes were then hybridized onto an arrayed slide. Fluorescence was captured with a GenePix4000B Scanner (Axon Instruments, Foster City, CA) and fluorescent intensities were quantified by using IMAGENE 5.5 (BioDiscovery, El Segundo, CA). All signal values, which were lower than the background level were treated as background and were excluded from calculations.

Clustering and visualization. DNA microarray data were K-mean clustered with CLUSTER (http://rana.lbl.gov/manuals/ClusterTreeView.pdf) and visualized using TreeView (4)**.**

Cloning of *E. coli* peroxiredoxin Tsa1. The gene coding for *E. coli* 2-Cys thiol peroxidase Ts1 was amplified with primers: 5'-gaaagactagtatgtcacaaaccgttcatttccag-3', and 5'-aggcatgtcgacttatgctttcagtacagccagag-3', and cloned into SpeI-SalI sites of p423 vector containing hygromycin resistance gene at the SacI site. The construct was transformed into WT cells and 7Δ cells, and the clones selected by resistance to 300 μg/ml hygromycin.

Estimation of average levels of gene activation and repression. Average values of gene activation and repression were estimated using the following equation:

$$S = \frac{\sum_{i=1}^{n} I}{n} - \frac{\sum_{j=1}^{k} (-D_j)}{k}$$

where N is the total number of yeast genes; I, the fold activation of genes that were induced at a minimum of two fold; n, the number of genes induced at a minimum of two fold; D, the fold repression for genes that were repressed at a minimum of two fold; and k, the number of genes repressed at a minimum of two fold. Fold activation and repression were calculated separately and their sum indicated the average value of gene expression changes. This equation was also used to calculate values for specific clusters and groups of genes; however, in that case, N was the number of genes in a cluster. This research and other computational analyses were completed utilizing the Prairiefire Beowulf cluster from Research Computing Facility of the University of Nebraska – Lincoln.

Identification of Yap1- and Yap1/Skn7-dependent genes. The dataset of gene expression response to H_2O_2 observed in mutants lacking individual or multiple oxidative stress transcription factors Yap1, Skn7, Msn2 and Msn4 was searched for the following expression patterns: (i) Yap1-dependent genes. Expression changes in Yap1, Yap1/Skn7 and quadruple 4Δ mutants should be a minimum of two fold lower than the expression changes in WT, Skn7 or Msn2/Msn4 mutant cells. (ii) Yap1/Skn7-dependent genes. Expression changes in Yap1, Skn7, Yap1/Skn7 and quadruple 4Δ mutants should be a minimum of two fold lower than the expression changes in WT and Msn2/Msn4 mutant cells.

Detection of reversibly oxidized cysteine residues in WT and mutant yeast strains. Each strain was grown in 200 ml of YPD to OD_{600} =1. 100 ml of each sample was removed, treated by 12% trichloroacetic acid (TCA), centrifuged, and washed with ice-cold 5% TCA. The remaining sample was treated with 0.5 mM H_2O_2, incubated for 1 min, and treated with TCA as described above. Each pellet was resuspended in 1 ml denaturing buffer (6 M urea, 200 mM Tris-HCl, pH 8.5, 10 mM EDTA, and 0.5% [w/v] SDS) containing 100 mM iodoacetamide. Iodoacetamide was used to alkylate all reduced cysteine residues. After 30 min of incubation at 25 ^oC, the reaction was stopped by the addition of ice-cold 12% TCA. The samples were incubated for 20 min on ice, centrifuged and washed with TCA as described above. The pellets were then dissolved in 1 ml of denaturing buffer containing 100 mM DTT and incubated for 30 min at 25 ^oC. Cysteine residues which were in the oxidized state in the initial sample and could not be alkylated by iodoacetamide were now in the reduced state. The reaction was stopped by addition of 12% TCA. After 20 min on ice, the samples were centrifuged and washed as described above. Three additional ice-cold acetone wash steps were performed to remove the remainder of DTT. The pellets were then dissolved in the denaturing buffer containing 0.5 μM biotinylated iodoacetamide (Molecular Probes) and incubated for 1 h at 25 ^oC. At this step, the cysteine residues which were oxidized in the initial sample were modified with biotin. Equal aliquots from each sample were resolved by SDS-PAGE, transferred onto a PVDF membrane, and proteins, in which cysteine residues were modified with biotin, were visualized by western blotting analysis using streptavidin-conjugated mouse antibodies.

Viability assays. Each strain was grown in 10 ml YPD to OD_{600} =0.5 and treated with indicated stress agents for various time periods. Cells were centrifuged, washed with YPD medium and serial dilutions were plated on YPD agar plates. Colony numbers were counted after 3 days of growth.

ROS analyses. Yeast cells were grown to OD_{600} 0.5, washed twice with PBS, resuspended in PBS to 10^8 cells/ml, and loaded with 5 μM DCFDA (Sigma). Cells were treated with 1 mM or 5 mM H_2O_2 for 30 min. DCF fluorescence was visualized by BD FACS Calibur (BD Biosciences) flow cytometer equipped with Sapphire Blue laser (488 nm) using 525 nm emission filter.

Genome sequence of 8Δ cells. Genomic DNA was isolated from 8Δ cells, fragmentized to 200 bp by sonication and sequenced on an Illumina platform at the University of Nebraska-Lincoln genomics facility. Reads were assembled into the genome with MAQ genome assembler.

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Legends to Supporting Figures

Figure S1. Verification of thiol peroxidase mutant strains. Detection of thiol peroxidase genes in the mutant strains by PCR is shown. Genomic DNA fragments were amplified using each thiol peroxidase gene specific primers. DNA from a WT strain was used as a positive control. This figure is spliced from many agarose gels. All bands were of the expected size as indicated on the left.

Figure S2. Growth of WT and thiol peroxidase mutant cells in the presence of indicated concentrations of (A) H_2O_2, (B) DTT, (C) diamide, and (D) menadione. Series of 10x dilutions of each strain were incubated on plates in the presence of indicated stress inductors for 48 h and the images were taken. A difference in growth between two single columns in the figure implies a 10-fold difference in survival.

Figure S3. A graph showing average values of gene activation and repression for wild type and thiol peroxidase mutant cells in response to 0.5 mM H_2O_2 treatment for 30 min. WT refers to BY4741 (isogenic with thiol peroxidase mutants) and WT1 refers to SY2626 (isogenic with Trx1,2Δ and Trr1Δ). Average values of activation and repression were estimated as described in Methods. WTa and 8Δa refer to experiments performed under anaerobic conditions.

Figure S4. Changes in gene expression of WT and indicated mutant thiol peroxidase strains in response to treatment with 0.5 mM H_2O_2 (left panel) and 0.1 mM H_2O_2 (right panel) are shown for all genes that are either activated or repressed in at least one strain (WT or mutant) used in the study. Numbers below the columns show average values of changes in gene expression (the sum of activation and modular repression values divided by the total number of yeast genes) for each dataset.

Figure S5. Average changes in gene expression in WT and 8Δ cells in response to 30 min treatment with different concentration of H_2O_2. Average values of gene activation and repression were determined as described in Methods.

Figure S6. Regulation of gene expression in response to H_2O_2 treatment in cytochrome c peroxidase and sulfiredoxin mutant strains. Right panel shows changes in gene expression between indicated mutant and WT cells.

Figure S7. Time course of changes in gene expression of rRNA modification and protein synthesis genes in WT and 7Δ cells in response to 0.5 mM H_2O_2, 2.5 mM DTT and 2.5 mM diamide. Gray shows spots with no data.

Figure S8. Regulation of expression of rRNA modification and protein synthesis genes. Changes in gene expression in response to H_2O_2 treatment are shown for thiol peroxidases mutants and isogenic wild type cells (left panel) and for Trr1 and Trx1/Trx2 mutant and isogenic wild type cells (middle panel). Right panel shows changes in gene expression between indicated mutant and isogenic WT strains. Gray shows spots with no data.

Figure S9. Regulation of gene expression of mitochondrial ribosomal proteins in response to H_2O_2 treatment (shown for thiol peroxidase mutants and isogenic WT cells). Gray shows spots with no data.

Figure S10. Regulation of gene expression of the ubiquitin-dependent protein degradation system in response to H_2O_2 (shown for thiol peroxidase mutant and isogenic WT cells). Gray shows spots with no data.

Figure S11. Regulation of gene expression in WT and 8Δ cells treated with 0.5 mM H_2O_2 for 30 min in the presence or absence of 5 mM proline (Pro) or 5 mM NAC, or under anaerobic conditions (shown with extension 'a'). Right panel shows changes in gene expression between indicated mutant and WT cells. Gray shows spots with no data.

Figure S12. (A) Detection of oxidized cysteine residues in yeast proteins in WT and indicated mutant strains. *E. coli* 2-Cys peroxiredoxin BCP was overexpressed in wild type and 7Δ cells, however, it did not change overall cysteine oxidation. (B) Amido Black staining of the gel shown in A.

Figure S13. Cysteine oxidation in yeast proteins in WT and 8Δ cells in the presence or absence of 5 mM NAC and 0.1 mM H_2O_2.

Figure S14. Growth curves of WT and 8Δ cells in YPD medium and in YPD supplemented with 3 mM NAC or 2 mM DTT.

Figure S15. Average values of gene induction and repression in response to 0.5 mM H_2O_2 treatment for WT cells and cells lacking four transcription factors that regulate gene expression in response to oxidative stress. The right area of the graph shows changes in gene expression between indicated mutant and WT cells. Average values of gene induction and repression were estimated as described in Materials and Methods.

Figure S16. Regulation of gene expression of cytosolic ribosomal proteins in response to H_2O_2 treatment. Left panel shows changes in gene expression for cytosolic ribosomal proteins upon treatment of WT cells and cells lacking four transcription factors with H_2O_2. Right panel shows changes in gene expression for cytosolic ribosomal proteins between indicated mutant and WT cells. To the right of this panel, ribosomal protein genes are shown.

Figure S17. Analysis of cysteine oxidation in yeast proteins in WT and indicated mutant strains.

Figure S18. Changes in expression of Yap1- and Yap1/Skn7-dependent genes in WT and 7Δ mutant cells in response to menadione, diamide and DTT treatments. Unlike menadione and diamide responses, the DTT response of Yap1- and Yap1/Skn7-dependent genes is opposite in WT and 8Δ cells. Gray shows spots with no data.

Figure S19. Regulation of gene expression by H_2O_2 and diamide. Changes in gene expression of WT and 8Δ strains in response to 0.5 mM H_2O_2 treatment (30 min), 2.5 mM diamide (30 min) or combined treatment are shown for all genes in yeast proteome. Numbers below the columns show average values of changes in gene expression

Figure S20. (A) Genes induced more than 2 fold in WT cells by H_2O_2 (897 genes), and induced by diamide in WT cells (1439 genes). These two groups have 787 genes in common. (B) Genes

repressed more than 2 fold in WT cells by H_2O_2 (923 genes), and repressed more than 2 fold by diamide in WT cells (1313 genes). These two groups have 790 genes in common.

Figure S21. (A) Genes induced more than 2 fold in WT cells by H_2O_2 (897 genes), and induced by diamide in 8Δ cells (1498 genes). These two groups have 756 genes in common. (B) Genes repressed more than 2 fold in WT cells by H_2O_2 (923 genes), and repressed more than 2 fold by diamide in 8Δ cells (1337 genes). These two groups have 767 genes in common.

Table S1. Yeast strains used in this study.

Strain	Designation of WT and mutant cells	Genotype	Source
BY4741	WT	MATa *his3 leu2 met15 ura3*	ATCC
GY8	Tsa1Δ	MATa *his3 leu2 met15 ura3 Δtsa1:KAN*	This study
GY9	Tsa2Δ	MATa *his3 leu2 met15 ura3 Δtsa2:LEU2*	This study
GY10	mPrxΔ	MATa *his3 leu2 met15 ura3 Δmtpx:URA3*	This study
GY11	nPrxΔ	MATa *his3 leu2 met15 ura3 Δntpx:MET15*	This study
GY12	Ahp1Δ	MATa *his3 leu2 met15 ura3 Δahp1:HIS3*	This study
GY14	5PrxΔ	MATa *his3 leu2 met15 ura3 Δtsa1:KAN, Δtsa2:LEU2, Δntpx:MET15, Δahp1:HIS3, Δmtpx:URA3*	Wong et al., 2004
GY15	GY15	MATα *his3 leu2 met15 ura3*	This study
GY22	Gpx1Δ	MATa *his3 leu2 met15 ura3 Δgpx1:KAN*	Avery et al., 2001
GY23	Gpx2Δ	MATa *his3 leu2 met15 ura3 Δgpx2:KAN*	Avery et al., 2001
GY24	Gpx3Δ	MATa *his3 leu2 met15 ura3 Δgpx3:KAN*	Avery et al., 2001
GY25	3GpxΔ	MATa *his3 leu2 met15 ura3 Δgpx1:URA3, Δgpx2:HIS3, Δgpx3:KAN*	Avery et al., 2001
GY29	7Δ (all PrxΔ+2 Gpx1,3Δ)	MATa *his3 leu2 met15 ura3 Δgpx1:URA3, Δgpx3:KAN, Δtsa1:KAN, Δtsa2:LEU2, Δntpx:MET15, Δahp1:HIS3, Δmtpx:URA3*	This study
GY30	GY30	MATα *his3 leu2 met15 ura3 Δgpx1:URA3, Δgpx2:HIS3, Δgpx3:KAN*	This study
GY100	8Δ (all PrxΔ+all GpxΔ)	MATa *his3 leu2 met15 ura3 Δtsa1:KAN, Δtsa2:LEU2, Δntpx:MET15, Δahp1:HIS3, Δmtpx:URA3, Δgpx2:HYG, Δgpx1:URA3, Δgpx3:KAN*	This study
GY101	6Δ (all PrxΔ+Gpx2Δ)	MATa *his3 leu2 met15 ura3 Δgpx1:URA3, Δgpx2:HYG, Δgpx3:KAN, Δtsa1:KAN, Δtsa2:LEU2, Δntpx:MET15, Δahp1:HIS3, Δmtpx:URA3*	This study
GY110	7Δ (all PrxΔ+2 Gpx1,2Δ)	MATa *his3 leu2 met15 ura3 Δgpx1:URA3, Δtsa1:KAN, Δtsa2:LEU2, Δntpx:MET15, Δahp1:HIS3, Δmtpx:URA3, Δgpx2:HYG*	This study
GY111	7Δ (3GpxΔ 4PrxΔ+Tsa1)	MATa *his3 leu2 met15 ura3 Δgpx1:URA3, Δgpx3:KAN, Δgpx2:HYG, Δtsa2:LEU2, Δntpx:MET15, Δahp1:HIS3, Δmtpx:URA3*	This study
GY70	Yap1Δ	MATa *his3 leu2 met15 ura3 Δyap1:KAN*	ATCC
GY71	Skn7Δ	MATa *his3 leu2 met15 ura3 Δskn7:KAN*	ATCC
GY72	Msn2Δ	MATa *his3 leu2 met15 ura3 Δmsn2:KAN*	ATCC
GY73	Yap1Δ Skn7Δ	MATa *his3 leu2 met15 ura3 Δyap1:KAN, Δskn7:LEU2*	This study
GY74	Msn2,4Δ	MATa *his3 leu2 met15 ura3 Δmsn2:KAN, Δmsn4:URA3*	This study
GY113	Sfp1Δ	MATa *his3 leu2 met15 ura3 Δsfp1:KAN*	ATCC
GY114	Ccp1Δ	MATa *his3 leu2 met15 ura3 Δccp1:KAN*	ATCC
GY115	Srx1Δ	MATa *his3 leu2 met15 ura3 Δsrx1:KAN*	ATCC
GY75	4Δ	MATa *his3 leu2 met15 ura3 Δyap1:KAN, Δskn7:LEU2, Δmsn2:HIS3, Δmsn4:URA3*	This study
SY2626	WT	MAT-a *ade2 ura3 leu2 trp1 his1 lys2*	G. Merrill (Oregon St.U.)
MY402	Trr1Δ	MAT-a *ade2 ura3 leu2 trp1 his3 lys2 Δ trr1:HIS3*	G. Merrill (Oregon St.U.)
MY405	Trx1,2Δ	MAT-a *ade2 ura3 leu2 trp1 his3 lys2 Δ trx1:LYS2, Δ trx2:LEU2*	G. Merrill (Oregon St.U.)

Figure S1

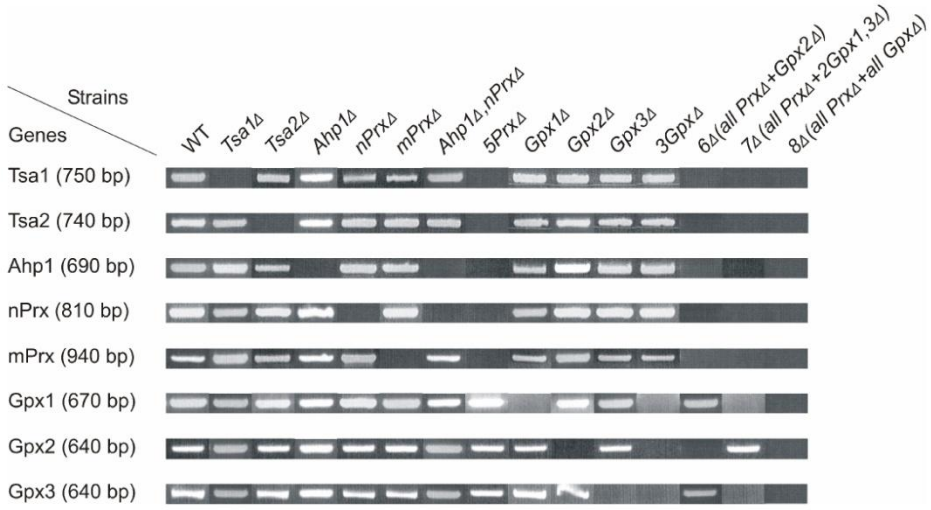

Strains
Genes
WT
Tsa1Δ
Tsa2Δ
Ahp1Δ
nPrxΔ
mPrxΔ
Ahp1Δ,nPrxΔ
5PrxΔ
Gpx1Δ
Gpx2Δ
Gpx3Δ
3GpxΔ
6Δ(all PrxΔ+Gpx2Δ)
7Δ (all PrxΔ+2Gpx1,3Δ)
8Δ(all PrxΔ+all GpxΔ)
Tsa1 (750 bp)
Tsa2 (740 bp)
Ahp1 (690 bp)
nPrx (810 bp)
mPrx (940 bp)
Gpx1 (670 bp)
Gpx2 (640 bp)
Gpx3 (640 bp)

Figure S2

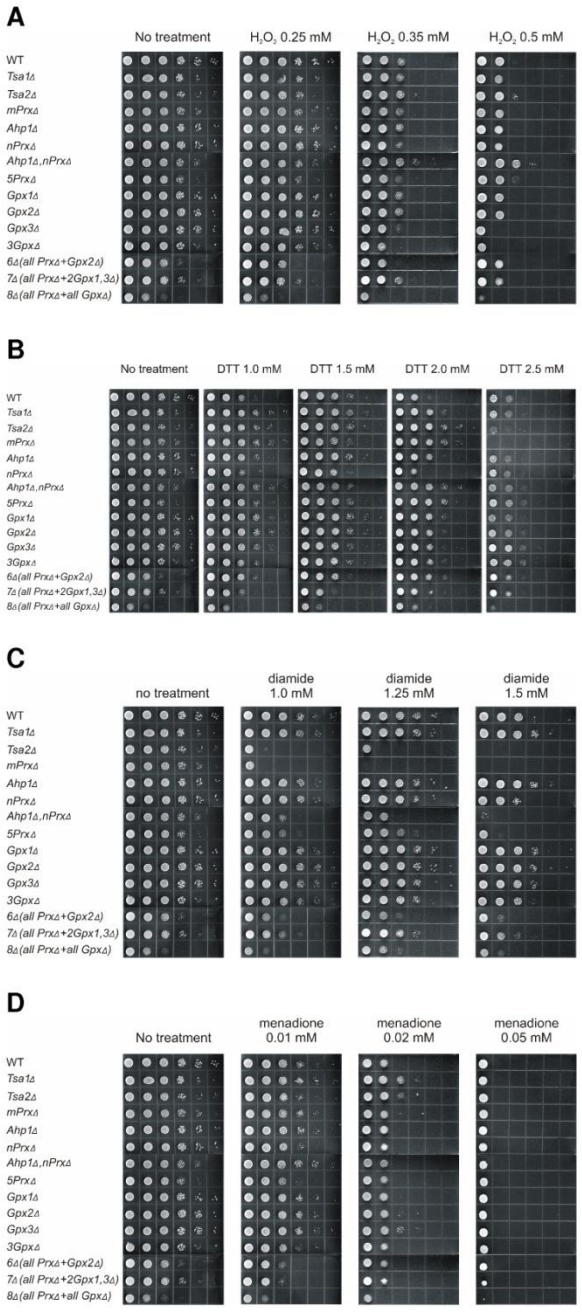

A
No treatment
H2O2 0.25 mM
H2O2 0.35 mM
H2O2 0.5 mM
WT
Tsa1Δ
Tsa2Δ
mPrxΔ
Ahp1Δ
nPrxΔ
Ahp1Δ,nPrxΔ
5PrxΔ
Gpx1Δ
Gpx2Δ
Gpx3Δ
3GpxΔ
6Δ(all PrxΔ+Gpx2Δ)
7Δ(all PrxΔ+2Gpx1,3Δ)
8Δ(all PrxΔ+all GpxΔ)
B
No treatment
DTT 1.0 mM
DTT 1.5 mM
DTT 2.0 mM
DTT 2.5 mM
C
no treatment
diamide 1.0 mM
diamide 1.25 mM
diamide 1.5 mM
D
No treatment
menadione 0.01 mM
menadione 0.02 mM
menadione 0.05 mM

Figure S3

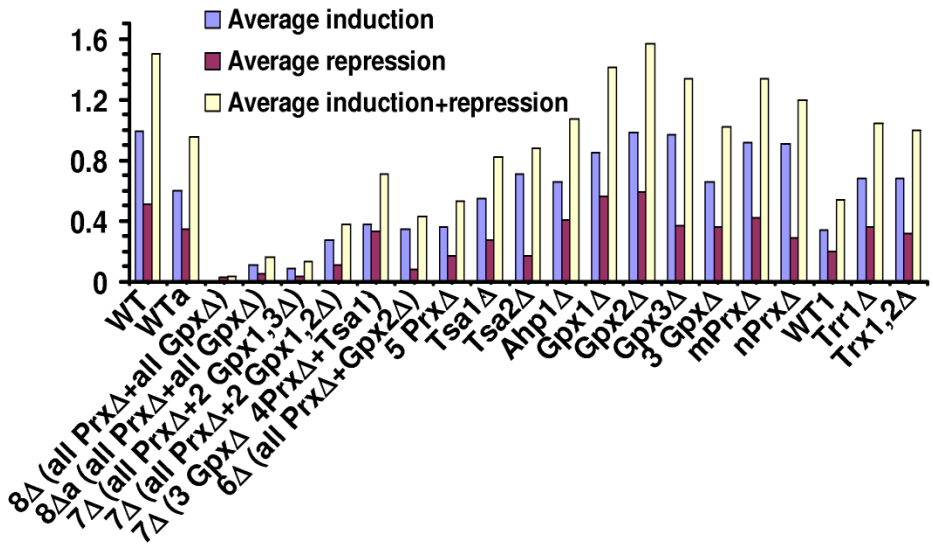

Average induction
Average repression
Average induction+repression
1.6
1.2
0.8
0.4
0
WT
WTa
8Δ (all PrxΔ+all GpxΔ)
8Δa (all PrxΔ+all GpxΔ)
7Δ (all PrxΔ+2 Gpx1,3Δ)
7Δ (all PrxΔ+2 Gpx1,2Δ)
7Δ (3 GpxΔ 4PrxΔ+Tsa1)
6Δ (all PrxΔ+Gpx2Δ)
5 PrxΔ
Tsa1Δ
Tsa2Δ
Ahp1Δ
Gpx1Δ
Gpx2Δ
Gpx3Δ
3 GpxΔ
mPrxΔ
nPrxΔ
WT1
Trr1Δ
Trx1,2Δ

Figure S4

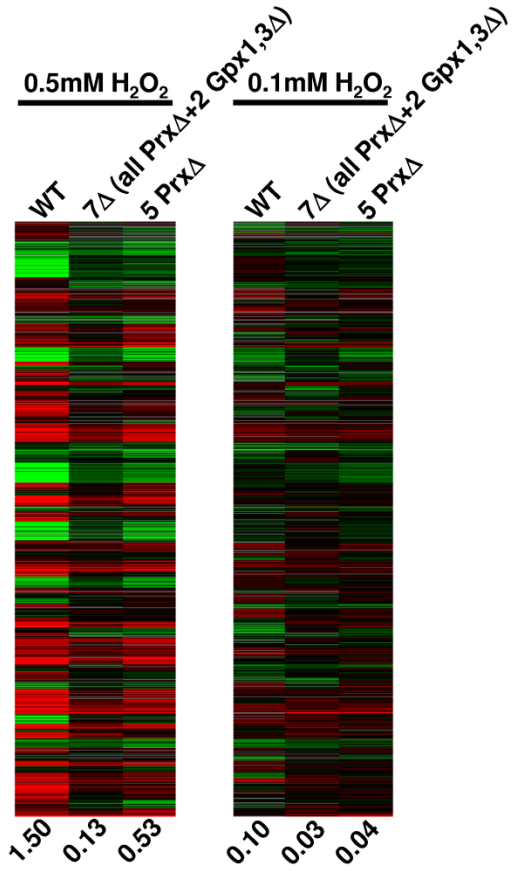

0.5mM H_2O_2
0.1mM H_2O_2
WT
7Δ (all PrxΔ+2 Gpx1,3Δ)
5 PrxΔ
WT
7Δ (all PrxΔ+2 Gpx1,3Δ)
5 PrxΔ
1.50
0.13
0.53
0.10
0.03
0.04

Figure S5

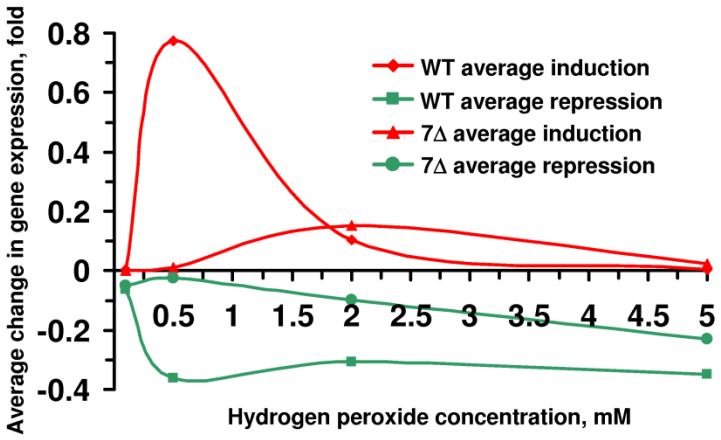

Average change in gene expression, fold
0.8
0.6
0.4
0.2
0
-0.2
-0.4
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Hydrogen peroxide concentration, mM
WT average induction
WT average repression
7Δ average induction
7Δ average repression

Figure S6

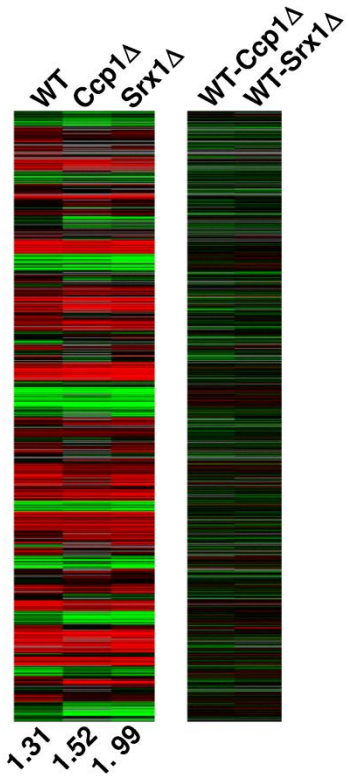


Figure S7

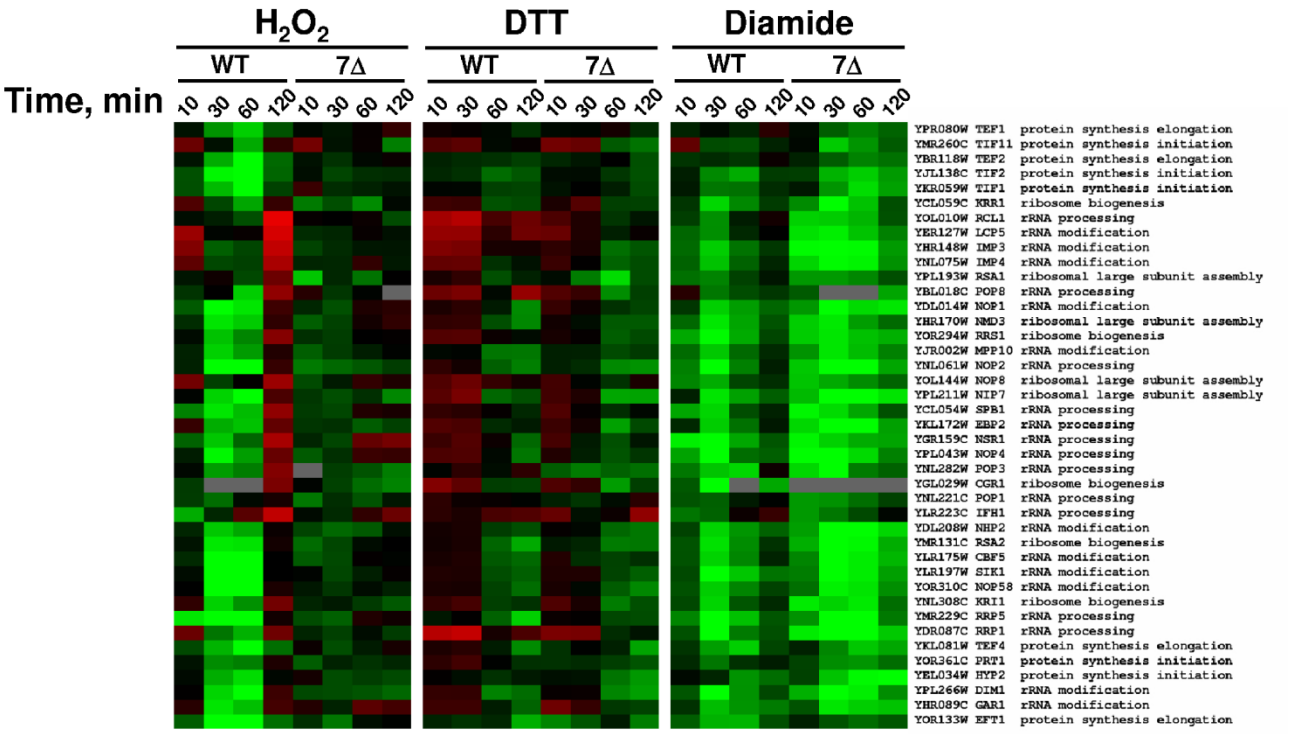

H2O2
DTT
Diamide
WT
7Δ
Time, min
10 30 60 120 10 30 60 120 10 30 60 120 10 30 60 120 10 30 60 120 10 30 60 120
YPR080W TEF1 protein synthesis elongation
YMR260C TIF11 protein synthesis initiation
YBR118W TEF2 protein synthesis elongation
YJL138C TIF2 protein synthesis initiation
YKR059W TIF1 protein synthesis initiation
YCL059C KRR1 ribosome biogenesis
YOL010W RCL1 rRNA processing
YER127W LCP5 rRNA modification
YHR148W IMP3 rRNA modification
YNL075W IMP4 rRNA modification
YPL193W RSA1 ribosomal large subunit assembly
YBL018C POP8 rRNA processing
YDL014W NOP1 rRNA modification
YHR170W NMD3 ribosomal large subunit assembly
YOR294W RRS1 ribosome biogenesis
YJR002W MPP10 rRNA modification
YNL061W NOP2 rRNA processing
YOL144W NOP8 ribosomal large subunit assembly
YPL211W NIP7 ribosomal large subunit assembly
YCL054W SPB1 rRNA processing
YKL172W EBP2 rRNA processing
YGR159C NSR1 rRNA processing
YPL043W NOP4 rRNA processing
YNL282W POP3 rRNA processing
YGL029W CGR1 ribosome biogenesis
YNL221C POP1 rRNA processing
YLR223C IFH1 rRNA processing
YDL208W NHP2 rRNA modification
YMR131C RSA2 ribosome biogenesis
YLR175W CBF5 rRNA modification
YLR197W SIK1 rRNA modification
YOR310C NOP58 rRNA modification
YNL308C KRI1 ribosome biogenesis
YMR229C RRP5 rRNA processing
YDR087C RRP1 rRNA processing
YKL081W TEF4 protein synthesis elongation
YOR361C PRT1 protein synthesis initiation
YEL034W HYP2 protein synthesis initiation
YPL266W DIM1 rRNA modification
YHR089C GAR1 rRNA modification
YOR133W EFT1 protein synthesis elongation

Figure S8

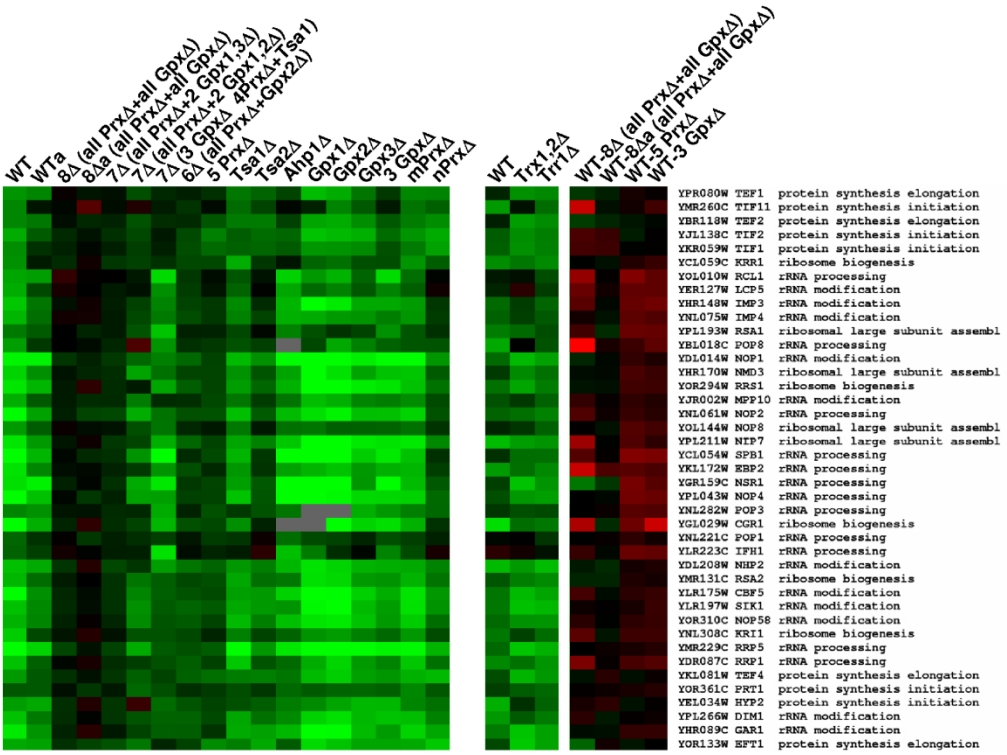

WT
WTa
8Δ (all PrxΔ+all GpxΔ)
8Δa (all PrxΔ+all GpxΔ)
7Δ (all PrxΔ+2 Gpx1,3Δ)
7Δ (all PrxΔ+2 Gpx1,2Δ)
7Δ (3 GpxΔ 4PrxΔ+Tsa1)
6Δ (all PrxΔ+Gpx2Δ)
5 PrxΔ
Tsa1Δ
Tsa2Δ
Ahp1Δ
Gpx1Δ
Gpx2Δ
Gpx3Δ
3 GpxΔ
mPrxΔ
nPrxΔ
WT
Trx1,2Δ
Trr1Δ
WT-8Δ (all PrxΔ+all GpxΔ)
WT-8Δa (all PrxΔ+all GpxΔ)
WT-5 PrxΔ
WT-3 GpxΔ
YPR080W TEF1 protein synthesis elongation
YMR260C TIF11 protein synthesis initiation
YBR118W TEF2 protein synthesis elongation
YJL138C TIF2 protein synthesis initiation
YKR059W TIF1 protein synthesis initiation
YCL059C KRR1 ribosome biogenesis
YOL010W RCL1 rRNA processing
YER127W LCP5 rRNA modification
YHR148W IMP3 rRNA modification
YNL075W IMP4 rRNA modification
YPL193W RSA1 ribosomal large subunit assembl
YBL018C POP8 rRNA processing
YDL014W NOP1 rRNA modification
YHR170W NMD3 ribosomal large subunit assembl
YOR294W RRS1 ribosome biogenesis
YJR002W MPP10 rRNA modification
YNL061W NOP2 rRNA processing
YOL144W NOP8 ribosomal large subunit assembl
YPL211W NIP7 ribosomal large subunit assembl
YCL054W SPB1 rRNA processing
YKL172W EBP2 rRNA processing
YGR159C NSR1 rRNA processing
YPL043W NOP4 rRNA processing
YNL282W POP3 rRNA processing
YGL029W CGR1 ribosome biogenesis
YNL221C POP1 rRNA processing
YLR223C IFH1 rRNA processing
YDL208W NHP2 rRNA modification
YMR131C RSA2 ribosome biogenesis
YLR175W CBF5 rRNA modification
YLR197W SIK1 rRNA modification
YOR310C NOP58 rRNA modification
YNL308C KRI1 ribosome biogenesis
YMR229C RRP5 rRNA processing
YDR087C RRP1 rRNA processing
YKL081W TEF4 protein synthesis elongation
YOR361C PRT1 protein synthesis initiation
YEL034W HYP2 protein synthesis initiation
YPL266W DIM1 rRNA modification
YHR089C GAR1 rRNA modification
YOR133W EFT1 protein synthesis elongation

Figure S9

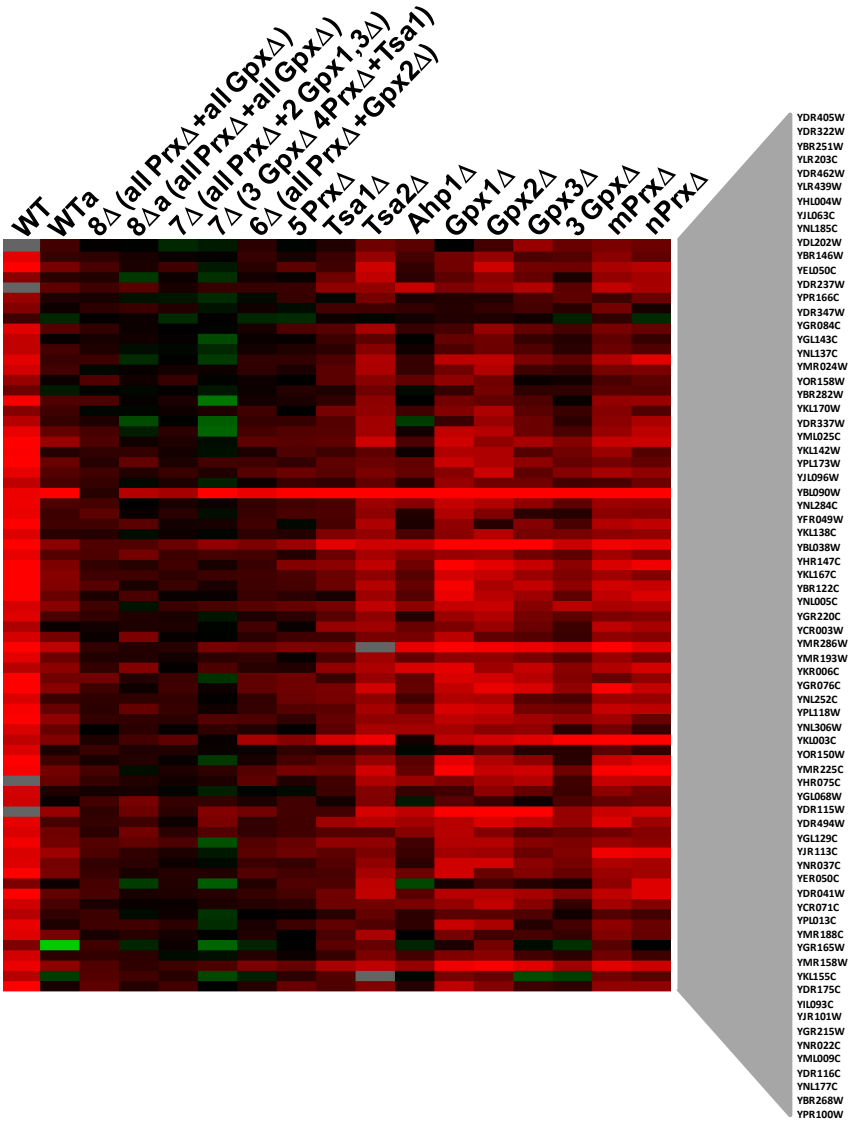

WT
WTa
8Δ (all PrxΔ+all GpxΔ)
8Δa (all PrxΔ+all GpxΔ)
7Δ (all PrxΔ+2 Gpx1,3Δ)
7Δ (3 GpxΔ 4PrxΔ+Tsa1)
6Δ (all PrxΔ+Gpx2Δ)
5 PrxΔ
Tsa1Δ
Tsa2Δ
Ahp1Δ
Gpx1Δ
Gpx2Δ
Gpx3Δ
3 GpxΔ
mPrxΔ
nPrxΔ
YDR405W
YDR322W
YBR251W
YLR203C
YDR462W
YLR439W
YHL004W
YJL063C
YNL185C
YDL202W
YBR146W
YEL050C
YDR237W
YPR166C
YDR347W
YGR084C
YGL143C
YNL137C
YMR024W
YOR158W
YBR282W
YKL170W
YDR337W
YML025C
YKL142W
YPL173W
YJL096W
YBL090W
YNL284C
YFR049W
YKL138C
YBL038W
YHR147C
YKL167C
YBR122C
YNL005C
YGR220C
YCR003W
YMR286W
YMR193W
YKR006C
YGR076C
YNL252C
YPL118W
YNL306W
YKL003C
YOR150W
YMR225C
YHR075C
YGL068W
YDR115W
YDR494W
YGL129C
YJR113C
YNR037C
YER050C
YDR041W
YCR071C
YPL013C
YMR188C
YGR165W
YMR158W
YKL155C
YDR175C
YIL093C
YJR101W
YGR215W
YNR022C
YML009C
YDR116C
YNL177C
YBR268W
YPR100W

Figure S10

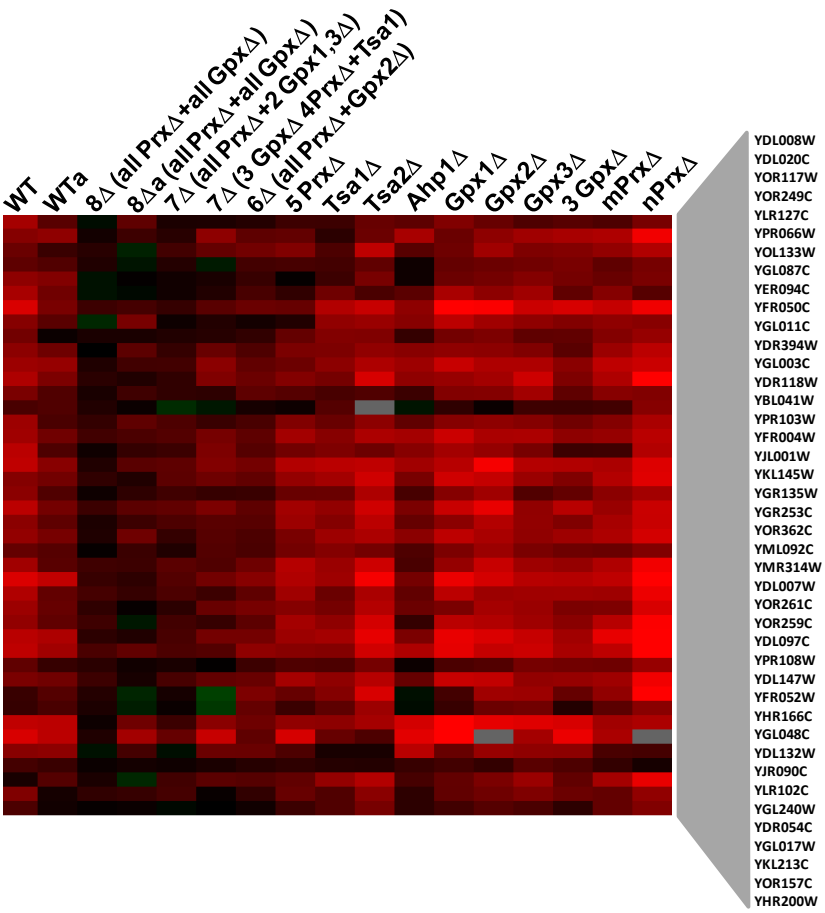

WT
WTa
8Δ (all PrxΔ+all GpxΔ)
8Δa (all PrxΔ+all GpxΔ)
7Δ (all PrxΔ+2 Gpx1,3Δ)
7Δ (3 GpxΔ 4PrxΔ+Tsa1)
6Δ (all PrxΔ+Gpx2Δ)
5 PrxΔ
Tsa1Δ
Tsa2Δ
Ahp1Δ
Gpx1Δ
Gpx2Δ
Gpx3Δ
3 GpxΔ
mPrxΔ
nPrxΔ
YDL008W
YDL020C
YOR117W
YOR249C
YLR127C
YPR066W
YOL133W
YGL087C
YER094C
YFR050C
YGL011C
YDR394W
YGL003C
YDR118W
YBL041W
YPR103W
YFR004W
YJL001W
YKL145W
YGR135W
YGR253C
YOR362C
YML092C
YMR314W
YDL007W
YOR261C
YOR259C
YDL097C
YPR108W
YDL147W
YFR052W
YHR166C
YGL048C
YDL132W
YJR090C
YLR102C
YGL240W
YDR054C
YGL017W
YKL213C
YOR157C
YHR200W

Figure S11

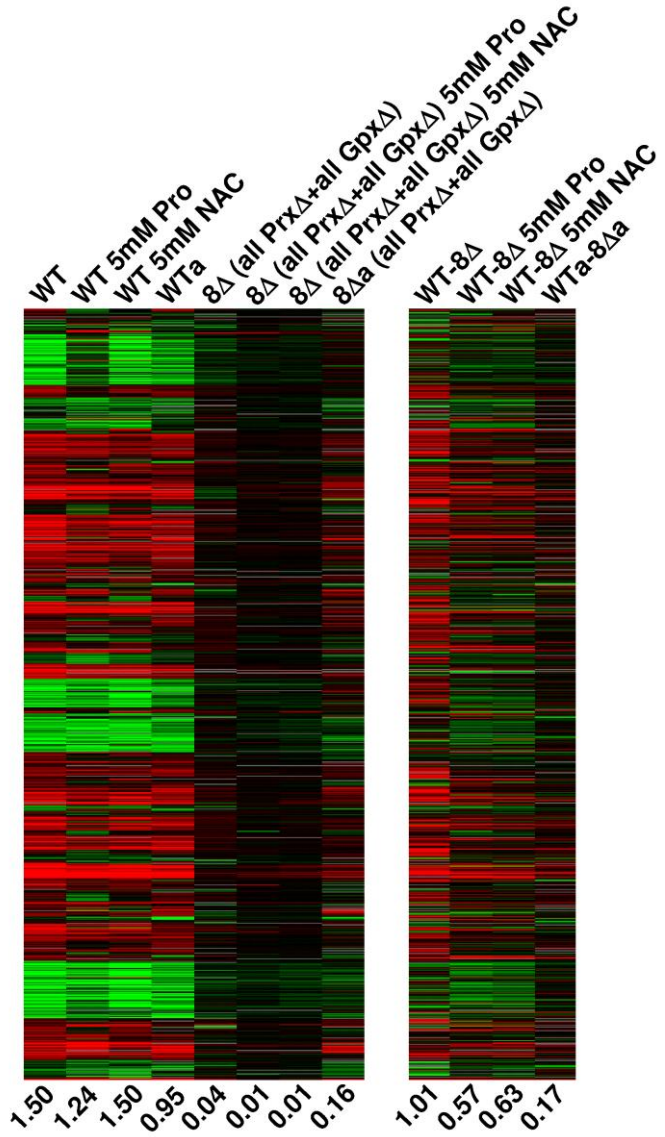

WT
WT 5mM Pro
WT 5mM NAC
WTa
8Δ (all PrxΔ+all GpxΔ)
8Δ (all PrxΔ+all GpxΔ) 5mM Pro
8Δ (all PrxΔ+all GpxΔ) 5mM NAC
8Δa (all PrxΔ+all GpxΔ)
WT-8Δ
WT-8Δ 5mM Pro
WT-8Δ 5mM NAC
WTa-8Δa
1.50
1.24
1.50
0.95
0.04
0.01
0.01
0.16
1.01
0.57
0.63
0.17

Figure S12

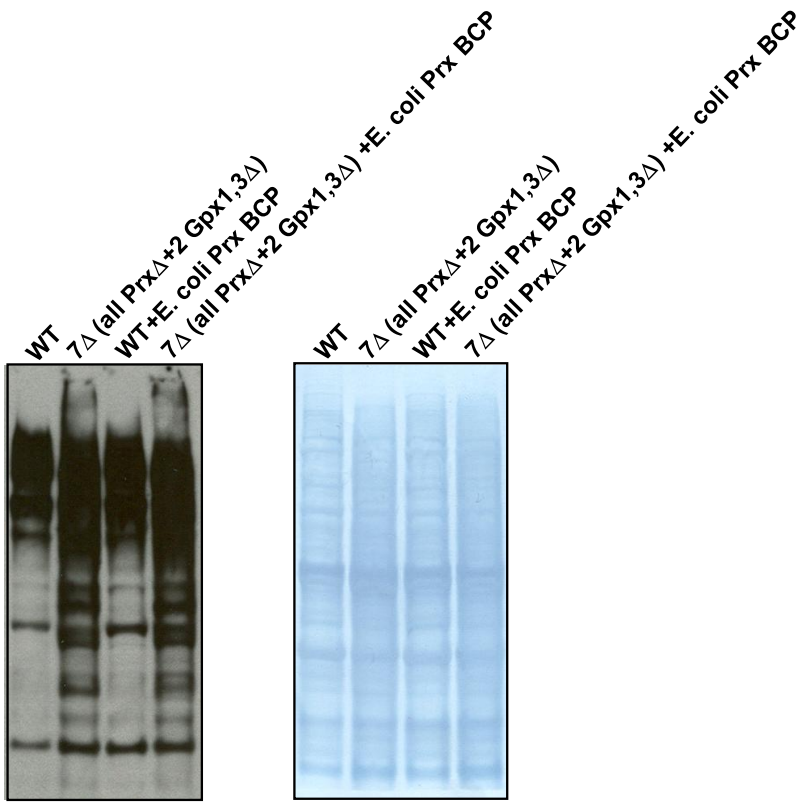

WT
7Δ (all PrxΔ+2 Gpx1,3Δ)
WT+E. coli Prx BCP
7Δ (all PrxΔ+2 Gpx1,3Δ) +E. coli Prx BCP
WT
7Δ (all PrxΔ+2 Gpx1,3Δ)
WT+E. coli Prx BCP
7Δ (all PrxΔ+2 Gpx1,3Δ) +E. coli Prx BCP

Figure S13

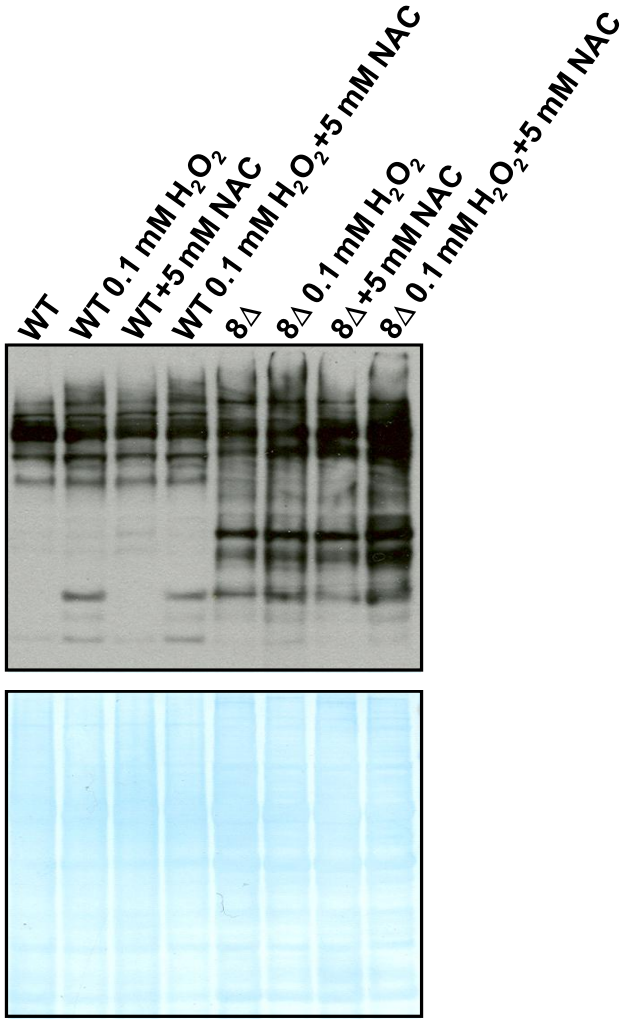

WT
WT 0.1 mM H_2O_2
WT+5 mM NAC
WT 0.1 mM H_2O_2+5 mM NAC
8Δ
8Δ 0.1 mM H_2O_2
8Δ+5 mM NAC
8Δ 0.1 mM H_2O_2+5 mM NAC

Figure S14

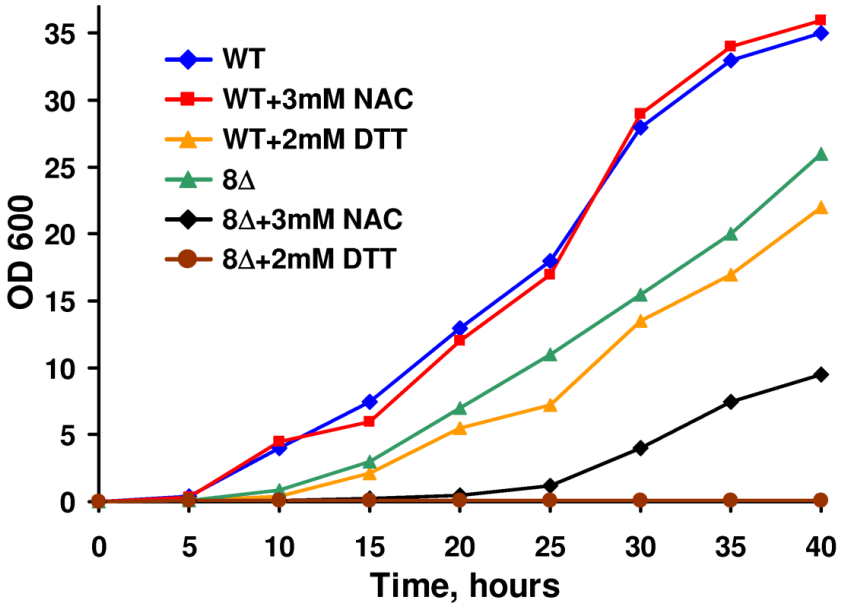

WT
WT+3mM NAC
WT+2mM DTT
8Δ
8Δ+3mM NAC
8Δ+2mM DTT
OD 600
Time, hours
0
5
10
15
20
25
30
35
40

Figure S15

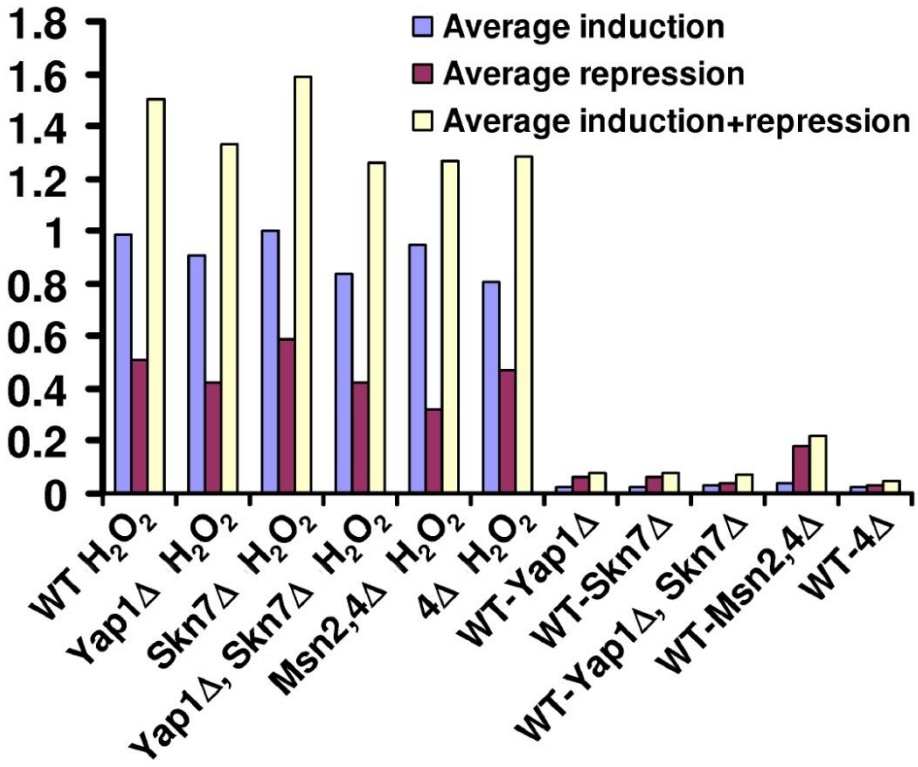

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average induction
Average repression
Average induction+repression
WT H2O2
Yap1Δ H2O2
Skn7Δ H2O2
Yap1Δ, Skn7Δ H2O2
Msn2,4Δ H2O2
4Δ H2O2
WT-Yap1Δ
WT-Skn7Δ
WT-Yap1Δ, Skn7Δ
WT-Msn2,4Δ
WT-4Δ

Figure S16

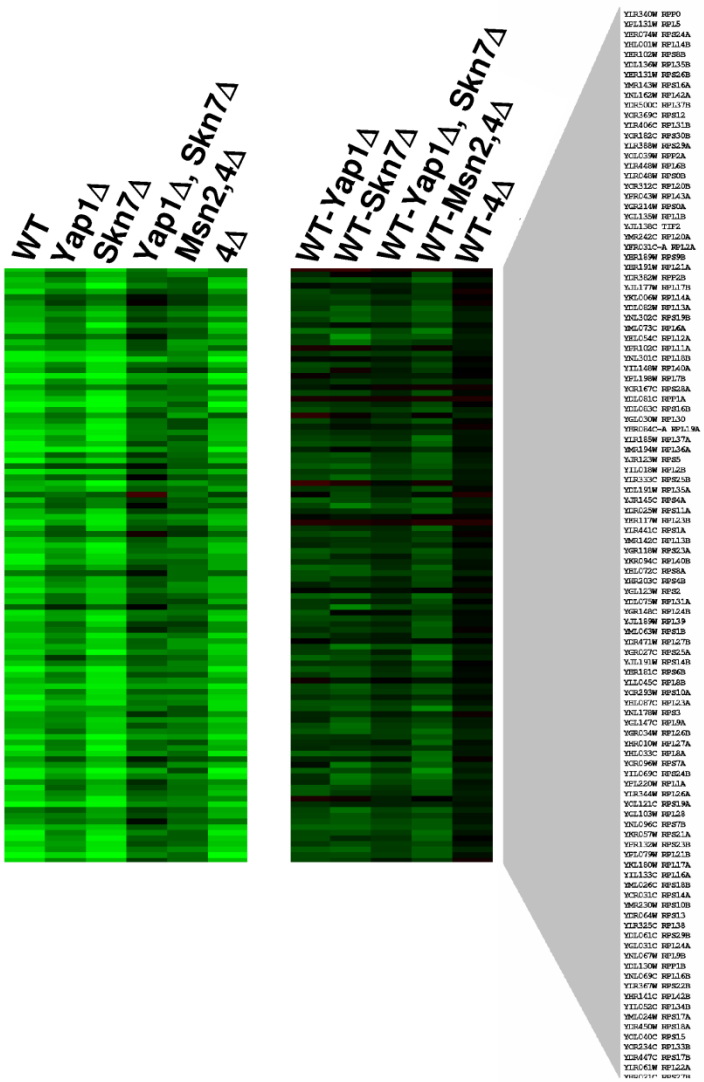

WT
Yap1Δ
Skn7Δ
Yap1Δ, Skn7Δ
Msn2,4Δ
4Δ
WT-Yap1Δ
WT-Skn7Δ
WT-Yap1Δ, Skn7Δ
WT-Msn2,4Δ
WT-4Δ

Figure S17

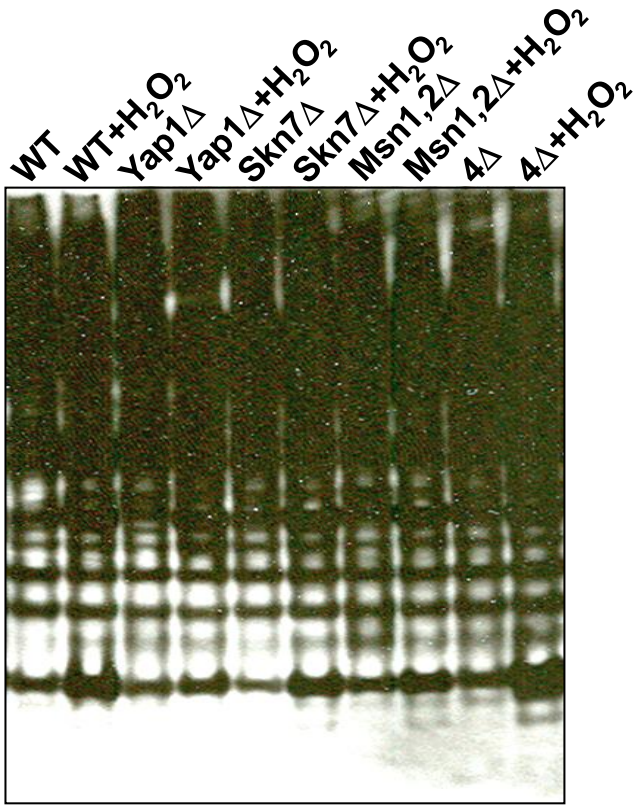

WT
WT+H2O2
Yap1Δ
Yap1Δ+H2O2
Skn7Δ
Skn7Δ+H2O2
Msn1,2Δ
Msn1,2Δ+H2O2
4Δ
4Δ+H2O2

Figure S18

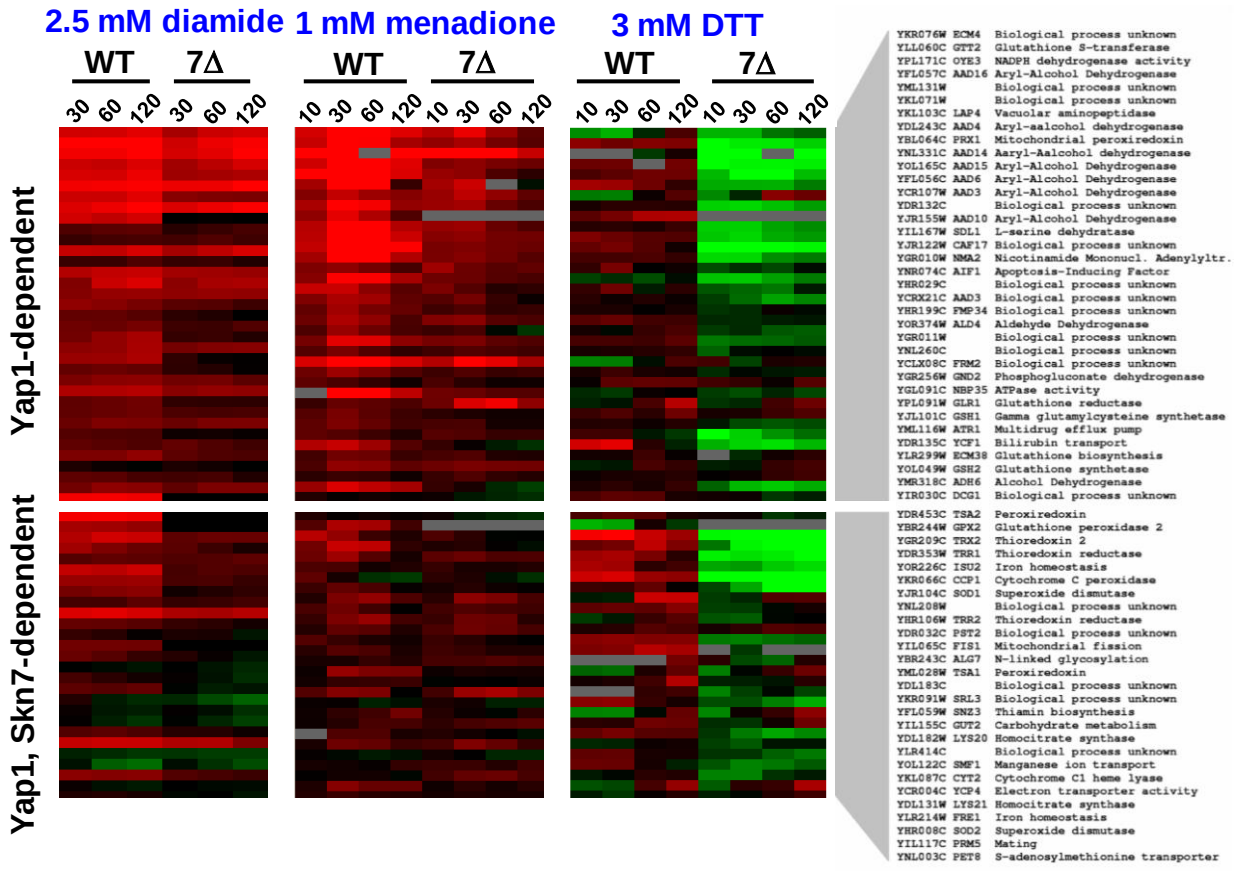

2.5 mM diamide
1 mM menadione
3 mM DTT
WT
7Δ
30 60 120 30 60 120
10 30 60 120 10 30 60 120
10 30 60 120 10 30 60 120
Yap1-dependent
Yap1, Skn7-dependent
YKR076W ECM4 Biological process unknown
YLL060C GTT2 Glutathione S-transferase
YPL171C OYE3 NADPH dehydrogenase activity
YFL057C AAD16 Aryl-Alcohol Dehydrogenase
YML131W Biological process unknown
YKL071W Biological process unknown
YKL103C LAP4 Vacuolar aminopeptidase
YDL243C AAD4 Aryl-aalcohol dehydrogenase
YBL064C PRX1 Mitochondrial peroxiredoxin
YNL331C AAD14 Aaryl-Aalcohol dehydrogenase
YOL165C AAD15 Aryl-Alcohol Dehydrogenase
YFL056C AAD6 Aryl-Alcohol Dehydrogenase
YCR107W AAD3 Aryl-Alcohol Dehydrogenase
YDR132C Biological process unknown
YJR155W AAD10 Aryl-Alcohol Dehydrogenase
YIL167W SDL1 L-serine dehydratase
YJR122W CAF17 Biological process unknown
YGR010W NMA2 Nicotinamide Mononucl. Adenylyltr.
YNR074C AIF1 Apoptosis-Inducing Factor
YHR029C Biological process unknown
YCRX21C AAD3 Biological process unknown
YHR199C FMP34 Biological process unknown
YOR374W ALD4 Aldehyde Dehydrogenase
YGR011W Biological process unknown
YNL260C Biological process unknown
YCLX08C FRM2 Biological process unknown
YGR256W GND2 Phosphogluconate dehydrogenase
YGL091C NBP35 ATPase activity
YPL091W GLR1 Glutathione reductase
YJL101C GSH1 Gamma glutamylcysteine synthetase
YML116W ATR1 Multidrug efflux pump
YDR135C YCF1 Bilirubin transport
YLR299W ECM38 Glutathione biosynthesis
YOL049W GSH2 Glutathione synthetase
YMR318C ADH6 Alcohol Dehydrogenase
YIR030C DCG1 Biological process unknown
YDR453C TSA2 Peroxiredoxin
YBR244W GPX2 Glutathione peroxidase 2
YGR209C TRX2 Thioredoxin 2
YDR353W TRR1 Thioredoxin reductase
YOR226C ISU2 Iron homeostasis
YKR066C CCP1 Cytochrome C peroxidase
YJR104C SOD1 Superoxide dismutase
YNL208W Biological process unknown
YHR106W TRR2 Thioredoxin reductase
YDR032C PST2 Biological process unknown
YIL065C FIS1 Mitochondrial fission
YBR243C ALG7 N-linked glycosylation
YML028W TSA1 Peroxiredoxin
YDL183C Biological process unknown
YKR091W SRL3 Biological process unknown
YFL059W SNZ3 Thiamin biosynthesis
YIL155C GUT2 Carbohydrate metabolism
YDL182W LYS20 Homocitrate synthase
YLR414C Biological process unknown
YOL122C SMF1 Manganese ion transport
YKL087C CYT2 Cytochrome C1 heme lyase
YCR004C YCP4 Electron transporter activity
YDL131W LYS21 Homocitrate synthase
YLR214W FRE1 Iron homeostasis
YHR008C SOD2 Superoxide dismutase
YIL117C PRM5 Mating
YNL003C PET8 S-adenosylmethionine transporter

Figure S19

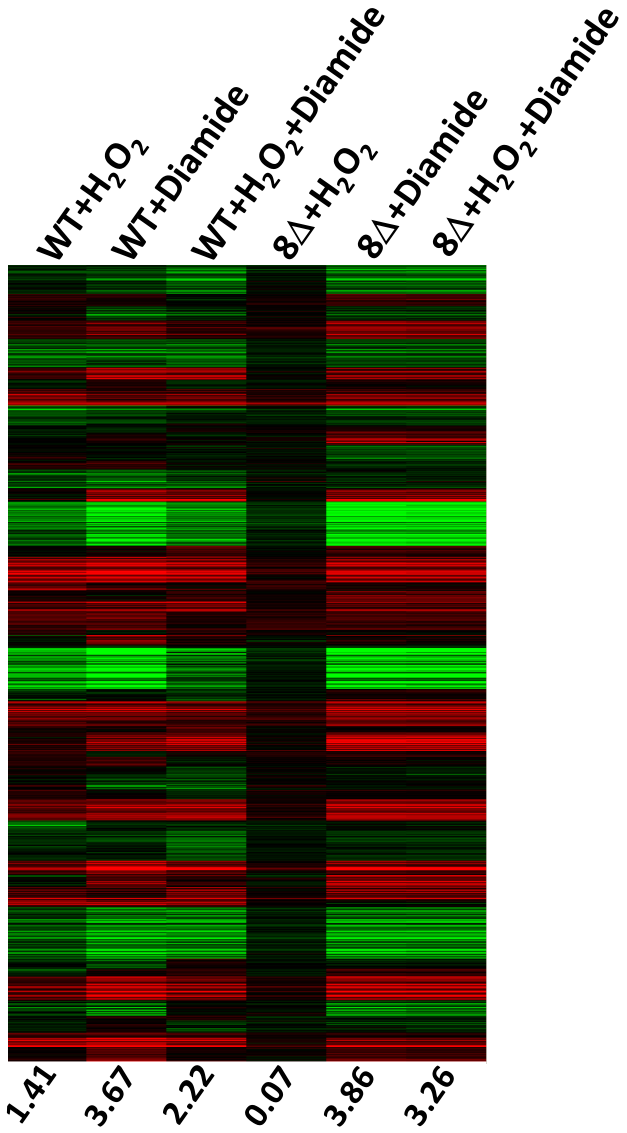

WT+H2O2
WT+Diamide
WT+H2O2+Diamide
8Δ+H2O2
8Δ+Diamide
8Δ+H2O2+Diamide
1.41
3.67
2.22
0.07
3.86
3.26

Figure S20

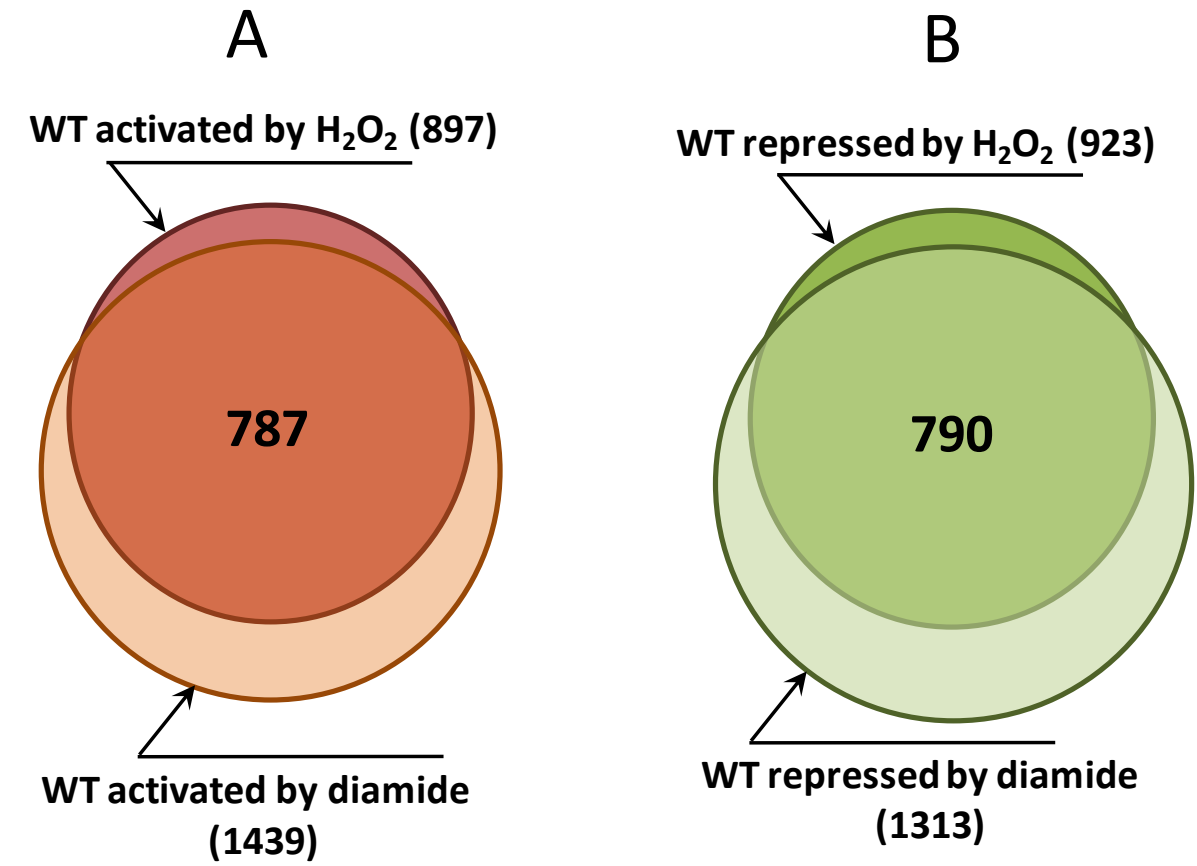
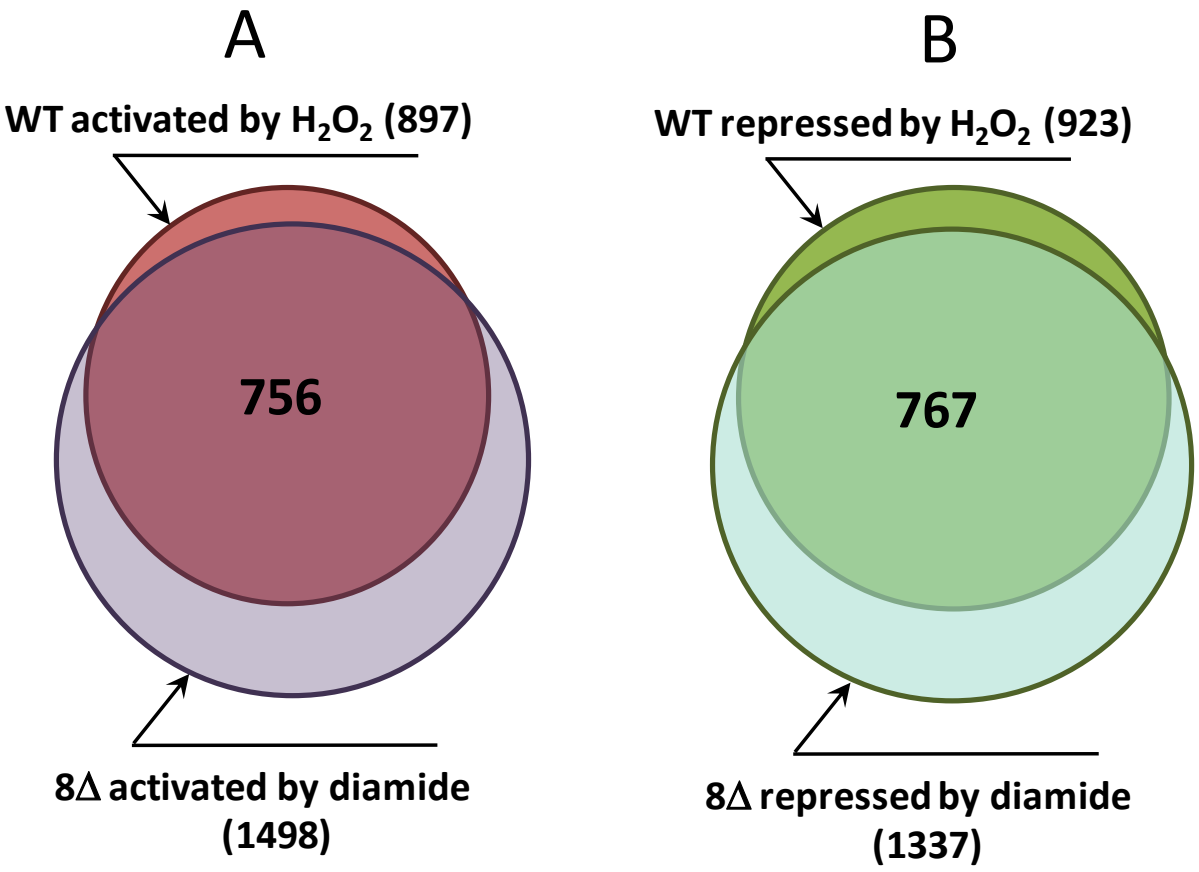

A
WT activated by H_2O_2 (897)
787
WT activated by diamide (1439)
B
WT repressed by H_2O_2 (923)
790
WT repressed by diamide (1313)

Figure S21


A
WT activated by H_2O_2 (897)
756
8Δ activated by diamide
(1498)
B
WT repressed by H_2O_2 (923)
767
8Δ repressed by diamide
(1337)